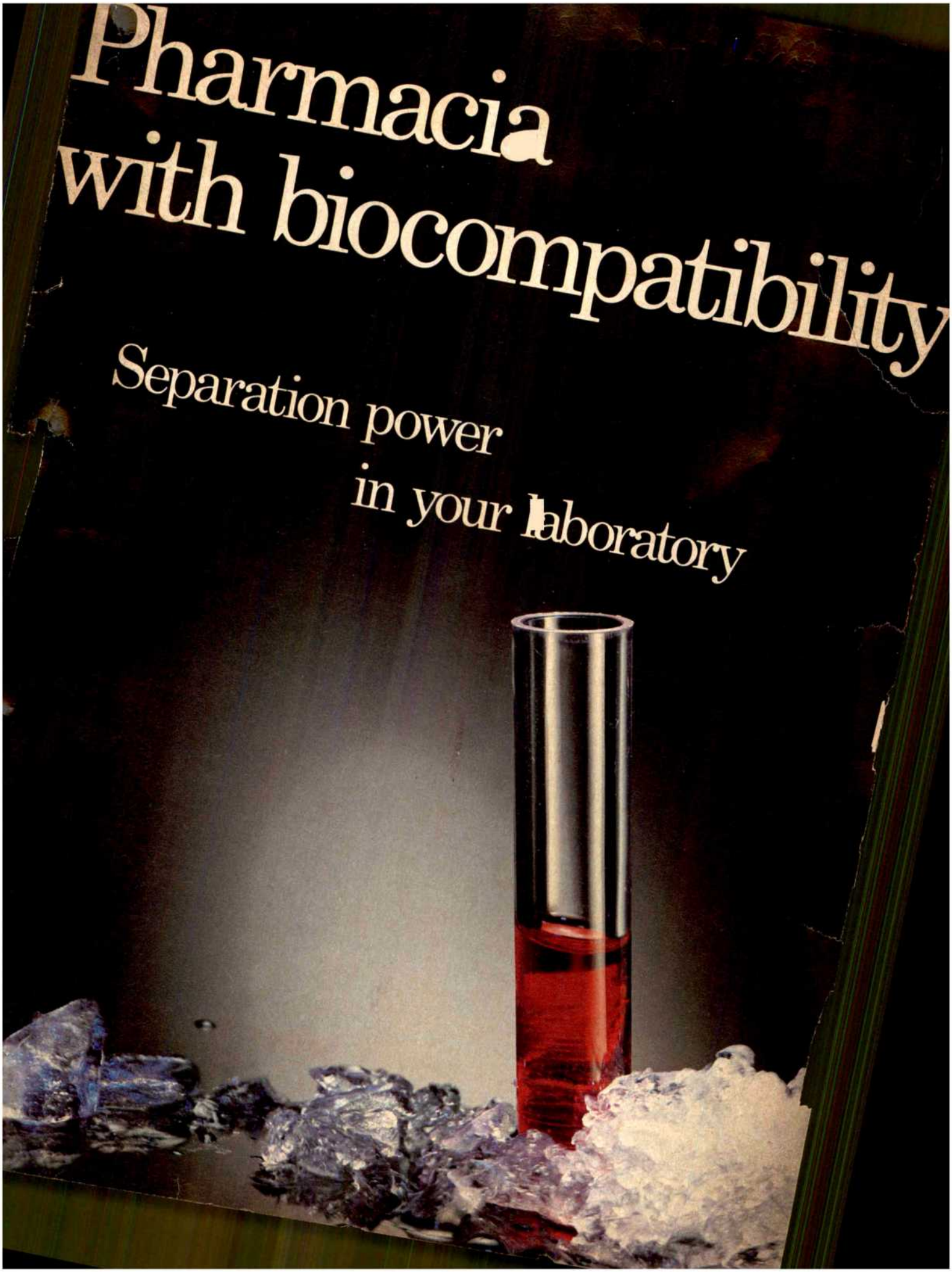

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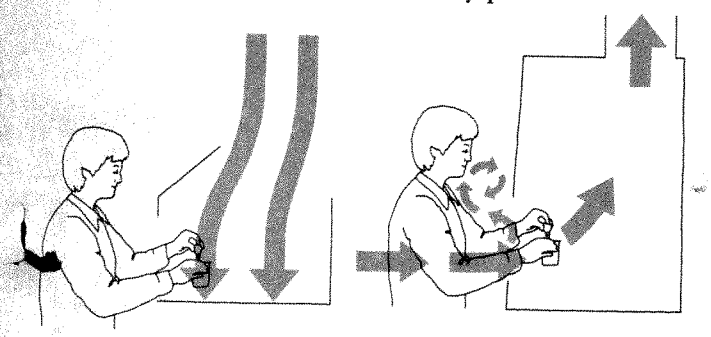
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Diagram 1

Leakage from fume cupboard with analyst at work. Measurements conducted by Dr. Sc. Bengt Ljungkvist of The Royal Institute of Technology, Stockholm, using the prescribed air flow, 600 cu. metres per hour and with the sash in the correct safety position with a 27 cm opening.

Diagram 2

Leakage from exhaust ventilated bench with analyst at work. The bench is from Innovent. Diagrams 1 and 2 are comparable. The conditions under which the measurements were carried out were similar. Measurements conducted by Dr. Sc. Bengt Ljungkvist of The Royal Institute of Technology, Stockholm with an air flow of 300 cu. metres per hour.

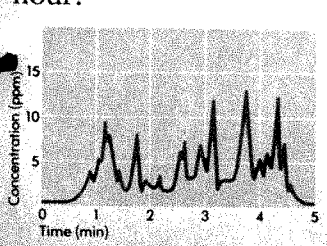


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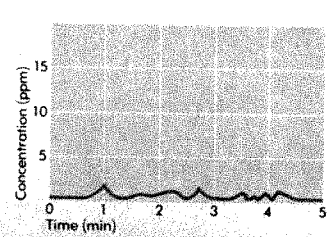


Diagram 2

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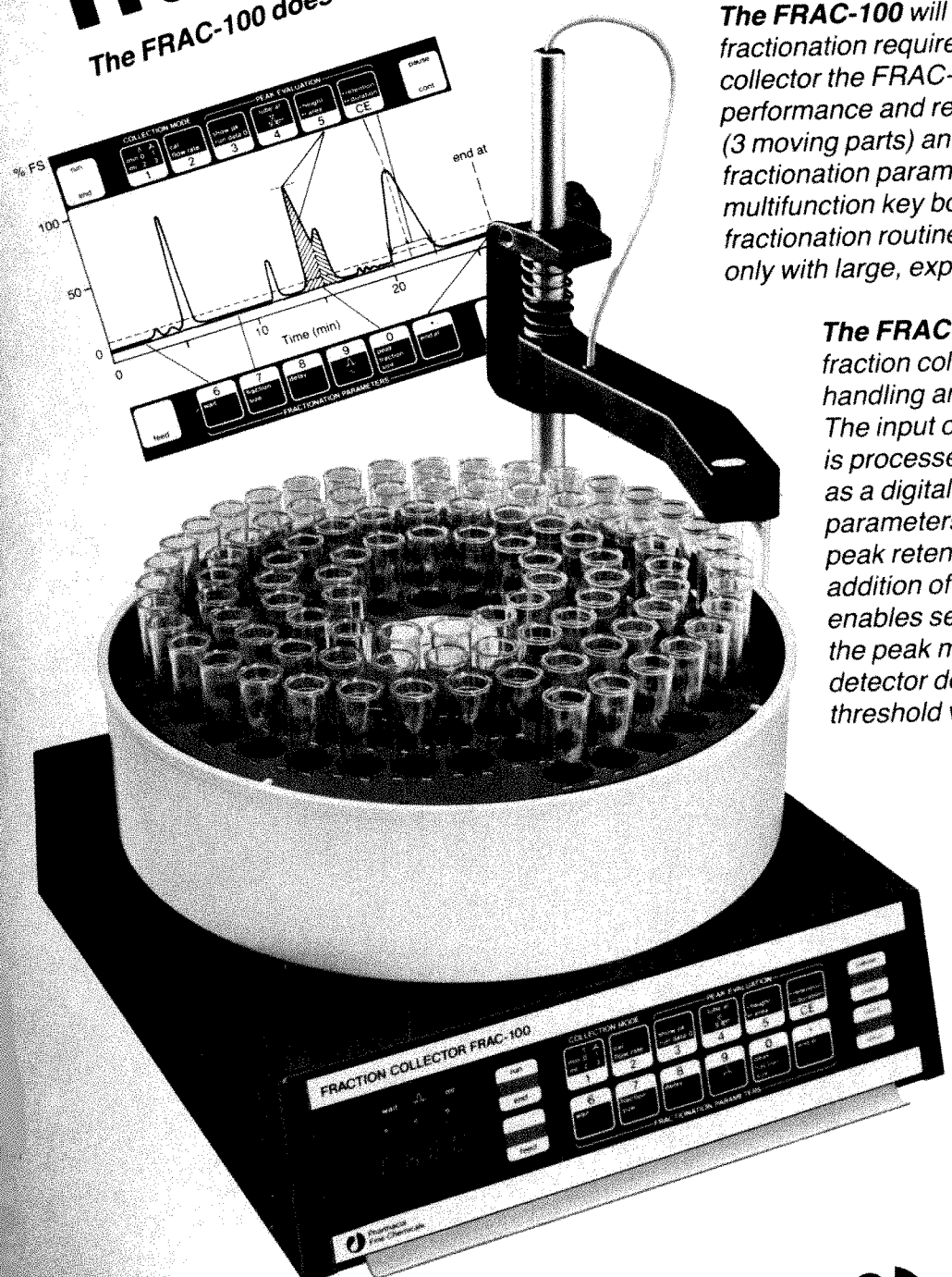
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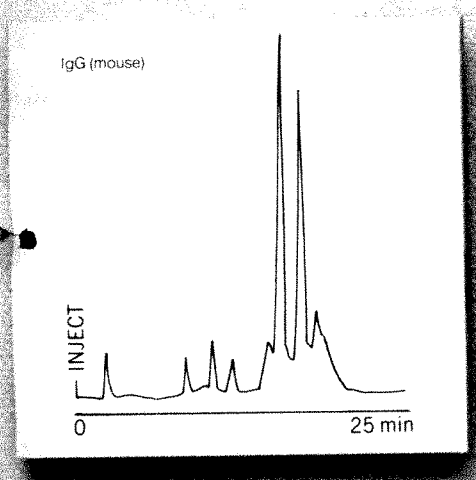
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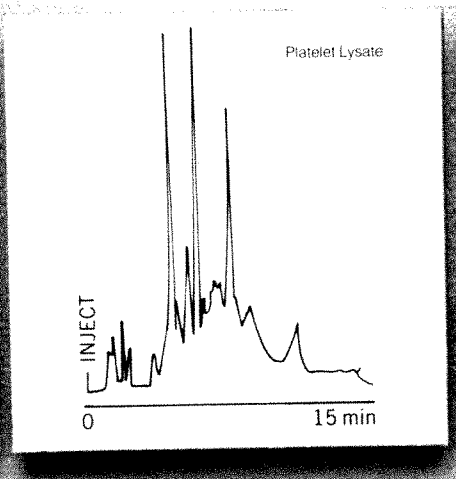
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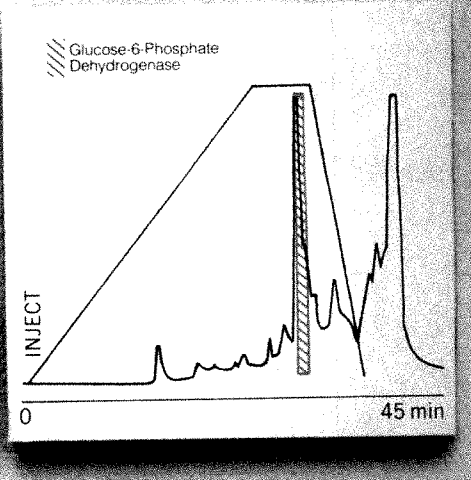
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September 10-12, 1984
Boston Park Plaza Hotel

Daily Themes:

DAY 1 - ONCOGENES,
DAY 2 - THE CANCER CELL,
DAY 3 - PROSPECTS/PROBLEMS.

MONDAY, 10 September
Exhibition Viewing: 8:30am — 6:30pm
Recruitment Center: 9:30am — 6:00pm

DAY 1
ONCOGENES AND GROWTH FACTORS — WHAT THEY ARE AND WHAT THEY DO

9:00 — 9:15	**Chairman**
9:15 — 10:00	Oncogenes *R. Weinberg (MIT)*
10:00 — 10:45	Growth factors *M. Spom (NIH)*
11.15 — 12.00	HTLV *R. Gallo (NIH)*
12.00 — 12.30	Clinician's view *S. Broder (NIH)*
2.00 — 4.00	Round table with morning speakers
4.30 — 5.30	The cancer cell *J. Cairns (Harvard)*

TUESDAY, 11 September
Exhibition Viewing: 8:30am — 6:30pm
Recruitment Center: 8:30am — 6:00pm

DAY 2
THE CANCER CELL — HEREDITARY CANCER, TUMOR PROMOTERS AND CARCINOGENESIS

9.00 — 9.15	**Chairman**
9.15 — 10.00	Gene regulation *P. Leder (Harvard)*
10.00 — 10.45	Hereditary cancer *R. White (Utah)*
11.15 — 12.00	Carcinogens and promoters *I.B. Weinstein (Columbia)*
12.00 — 12.30	Clinician's view *F. Gilbert (Mt Sinai)*
2.00 — 4.00	Round table with morning speakers
4.30 — 5.30	NCI program

WEDNESDAY, 12 September
Exhibition Viewing: 8:30am — 3:00pm
Recruitment Center: 8:30am — 3:00pm

DAY 3
PROSPECTS AND PROBLEMS — DRUG RESISTANCE, MONOCLONAL ANTIBODIES AND NEW DRUGS

9.00 — 9.15	**Chairman**
9.15 — 10.00	Diagnostics and therapeutics *G. Todaro (Oncogen)*
10.00 — 10.45	Immunotoxins *J. Uhr (Texas)*
11.15 — 12.00	Drug resistance *A. Varshavsky (MIT)*
12.00 — 12.30	Clinician's view *J. Gutterman (Houston)*
2.00 — 4.00	Round table with morning speakers
4.00 — 4.45	Prospects
4.45	Closing remarks

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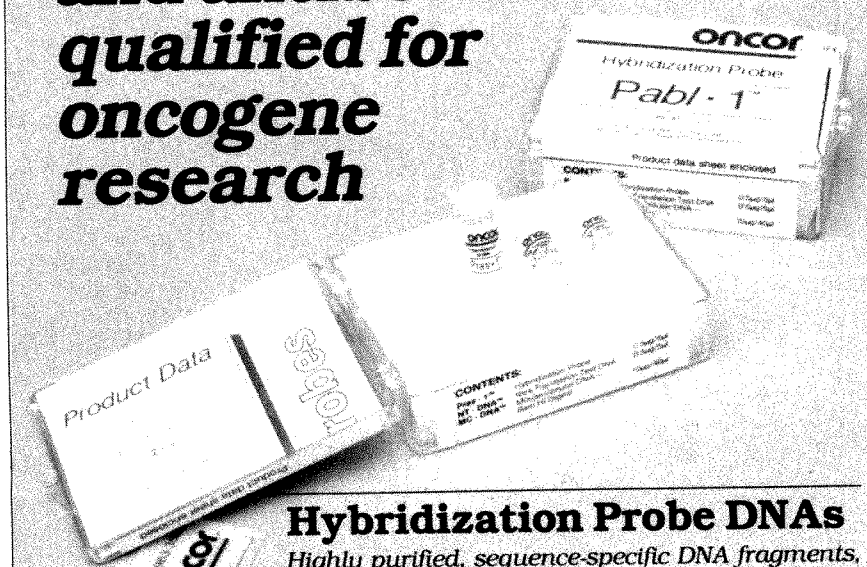
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nature

Attrition, not conquest, of disease

The AIDS saga has been a nasty shock for people accustomed to take modern medicine for granted. The discovery of the virus responsible should remind them of the enduring role of medical research.

AIDS (acquired immune deficiency syndrome) has engendered high drama from the beginning, a mere three years ago. Not every day does a new disease entity pop up, apparently from nowhere. Nor does it often happen that the main foci of a new disease are not in some far-off and half-forgotten developing country but in the most industrially advanced communities in the world. AIDS has shaken the complacency of hosts of people in no present danger of becoming afflicted.

The relatively small numbers of those suffering overtly from AIDS (thousands in the United States, hundreds in Western Europe) have been counterbalanced in people's imaginations by the bizarre features of the condition. People almost always die, but from one of several secondary causes. Most patients have been homosexual men, but women and their newborn children as well as people receiving blood transfusions and factor VIII (in haemophilia treatment) have been affected. This has raised alarm that whatever agent may be responsible is confined to those now afflicted only fortuitously — and temporarily. The notion, increasingly plausible in recent months, that the agent responsible is most probably a virus has been particularly shocking for smug health-ridden societies long since persuaded that medicine is a routine technology, even a physicians' ramp, and that good health is either a consumer purchase or an attitude of mind. Was not infection abolished with penicillin, anxious people have been complaining?

The latest instalment of the AIDS drama should give the lie to many of these nonsensical expectations. AIDS is almost certainly caused by a virus, that most clearly characterized by Robert Gallo and his associates at the National Cancer Institute in the United States, but with a high chance that the virus previously described by Luc Montagnier at the Pasteur Institute in Paris is identical. Both are retroviruses carrying genetic information as RNA, a DNA version of which becomes incorporated into the genes of infected cells. The most telling evidence is that a majority of the patients with AIDS carry antibodies against one of the viral proteins — at some earlier stage, their immune systems have responded to an infection which has nevertheless overwhelmed a large proportion of them. But as yet, little is known of the mechanism of infection, of the reasons why one class of T cells among lymphocytes is put out of action by the virus or of the status of those who have already been infected (to judge from the antibodies they carry) but who have not developed symptoms of AIDS. (The haunting question is whether they are immune or destined to run into trouble.) And next to nothing is known of the natural history of the virus. So why, the sceptics will be asking, is there all this fuss? If neither Gallo nor Montagnier can offer a cure, what benefit is there in what they have accomplished?

Virus

The simplest answer is also primitive. AIDS, a mystery only a few weeks ago, has been transformed into another virus disease. And that transformation, even though it will not by itself prevent the death of a single patient, has the utmost practical importance. Correspondents this week (p.12) and last have explained what steps can now in principle be taken — the identification of those among the sexual partners of AIDS patients who have been exposed to the virus (and who may or may not be carriers or at risk themselves), the screening of transfusion blood (if practicable) and so on. The essential tools for these investigations are the standard techniques of pre-molecular immunology. So AIDS has been lumped in the same category as, say, measles.

The same techniques will play a crucial part in helping to unravel the natural history of the disease (and the suspected link with central Africa) — as will the mere knowledge that a virus is indeed responsible. The only practical benefit certain to flow from what will now happen is that some people at risk will be helped to avoid infection. But the conversion of a mystery into a medical problem will also provide people with a sense of confidence that would otherwise not have been supportable. What has happened with AIDS is yet another illustration of the well-known truth that to know the cause of a problem is a necessary part of its solution.

Control

But when will there be a vaccine to prevent the spread of AIDS, or even a way of curing those already infected? That is what public health officials need, and what many of their constituents will insist can alone count as progress. The again maddeningly simple answer is that nobody can tell. It has taken fifteen years from the recognition of the then-surprising life-cycle of the hepatitis-B virus to the development of vaccines (now going into trials) whose efficacy is not yet known. AIDS is only the second human disease known to be associated with a retrovirus — the other is adult T-cell leukaemia, first recognized in south-east Japan — and the obvious ways of going about the job of vaccinating against AIDS could yet prove ineffective. The prospects for more effective treatment for patients already stricken are even less certain (although not necessarily more distant). But when the causes of death in AIDS are the accidental infections patients pick up and the otherwise rare skin cancer called Kaposi's sarcoma, and if the reasons for these developments are the known deficiency of helper T cells in patients' blood, the only guarantor of more effective treatment is the better understanding of the human immune system at which literally hundreds of laboratories have set their sights. (It will be sheer good luck if the trials with the genetically engineered humoural agent interleukin-2 also now being mounted turn out to be successful.) Like it or not, those who look for quick results must cultivate patience instead.

Those to whom that advice is unpalatable should reflect that things might be much worse. Montagnier's observations, reported last month (*The Lancet* **i**, 753; 1984), turned on the lucky accident that of two haemophiliac brothers being treated with factor VIII, only one had developed overt AIDS when first seen. Gallo's investigation, based on samples from many more patients, was a natural extension of his group's interest going back for four years in human T-cell leukaemia virus, type I of which may or may not be the same as that responsible for adult T-cell leukaemia in Japan. Here again, however, it is good luck that Gallo's group is now well placed to apply to the further definition of the AIDS virus the techniques of genetic manipulation being vigorously applied to the understanding of the role of retroviruses and oncogenes in carcinogenesis. In other words, the understanding of AIDS that has now been wrung from a seemingly obdurate problem is striking proof of the value of the basic biomedical research on which people have for decades enthusiastically lavished their talents — and governments, less

sure of themselves, their resources.

In these terms, rapid demonstration that AIDS is indeed caused by a virus is a triumph, even if not quite the "triumph over disease" that Mrs Margaret Heckler, the US Secretary of Health, described it last week. The triumph is that the research community has been able to respond so quickly to an unexpected development such as the emergence of AIDS. The explanation is again simple, exasperatingly so — the richness and diversity of the biomedical research enterprise, in the United States in particular but also throughout the world.

More good luck? Almost certainly not. The chances are high that in the present pattern of medical research, there would have emerged some group of workers somewhere in the world able — and willing — to turn from its long-term interests to throw light on a medical emergency such as that which cropped up three years ago. This is exactly what happened a decade ago with Legionnaires' disease, in its time also a threat to people's peace of mind. None of this diminishes the importance of what Montagnier and Gallo have separately done although, with both Legionnaires' disease and AIDS, the work done by the Centers for Disease Control at Atlanta, Georgia, deserves widely to be applauded. (So too does that of the clinicians who, in the past three years, have been required to treat AIDS patients with forms of therapy offering palliation only, who have in the process courageously taken to unprecedented lengths the openness and compassion that ideally characterize relationships between physicians and their patients and whose services in this regard will unhappily be required for many years to come.)

The moral, for Mrs Heckler as well as for the impatient sections of advanced communities eager to indulge the luxury of pretending that the practice and the pursuit of medicine have long since been replaceable by suitable doses of orange juice, physical exercise and mental application, is that the deliberate provision of medical care is in one important sense only a little more certain now than, say, a century ago. By means of large and costly social expenditures in research laboratories and clinics, the incidence of avoidable death is kept at a socially acceptable low level, but there is no avoiding the hazards still unknown. The best that can be done is to be prepared to deal with these hazards quickly, as they declare themselves. The case of AIDS has triumphantly illustrated the versatility of the biomedical research system patiently put in place in recent decades. Successive US governments have been especially farsighted in recognizing the importance of such a development. It is to be hoped that this latest emergency will help to win wider acceptance of the value of a vigorous research enterprise. For there is no prospect of a triumph over disease in general. □

Double-talk on animals

NIH seems more ready to risk its reputation than to meet serious critics on animal care.

ANTIVIVISECTIONISTS and other crusaders have never made intellectual integrity one of their top priorities. Appeals to emotion, however inconsistent or however specious, continue to be the hallmark of those who wish to halt animal experiments. So it is no accident that cute animals — preferably those with a lot of fur — star in the antivivisectionists' advertising campaigns (which have now reached the underground railway system in Washington, DC, in the form of posters of a child with a dog and a message that scientists who have perfectly reasonable alternatives to animal experimentation are hoping to make that dog suffer).

The National Institutes of Health (NIH), which support the majority of animal experiments in the United States, have now apparently decided that they too can play that game. At the first of what will be a series of meetings around the country, NIH produced for the public's heartstrings real-life patients and their families who have benefited from animal experiments. While the sincerity of what these patients had to say is indisputable, one has to feel uncomfortable at the exploitation of their misfortune inevitably at play when the federal government stands them up to say how much they love their husbands and how grateful they are that medical research has kept their children's daddies alive.

One has to feel even more uncomfortable at the nagging suspicion that NIH's simultaneous announcement of a new and supposedly tougher set of rules for the care and use of laboratory animals is nothing more than a part of this same public relations campaign. This conclusion can only be reinforced by NIH's simultaneous release of a study of animal experimentation at ten research institutions, chosen at random, that finds the old rules are working fine and that there are no problems to correct. If so, why correct them, unless the aim is to make a — very public — point?

In reality, the new regulations, which are published as a proposal open for public comment, do very little. Research institutions under the new regime will not only have to "commit themselves to implementing" animal care requirements — they will now have to "implement" them, a major crackdown if there ever was one. The only substantive change in the proposal is that the institutional animal care committees, charged with monitoring the use of laboratory animals at each research institution, would have to include one outside member.

NIH director James Wyngaarden set the tone for the NIH stance when, at last month's meeting, he declared his "deep concern" that public support for laboratory experiments involving animals may be eroding, the result of "politically sophisticated critics" who are attacking animal experiments as unnecessary and inhumane. NIH arguably have a role in public education to counter this trend, but need not sacrifice integrity in the process. If "political sophistication" means issuing meaningless regulations for the sake of appearances, NIH are better off with naivety. Or is it that by invoking the bogey of rabid antivivisectionists seeking to shut down biomedical research, NIH hope to distract attention from some serious proposals for reform (as opposed to revolution)?

It taxes credulity to claim that the extreme "animal rights" positions inherent in the antivivisectionists' stance are about to sway the overwhelmingly carnivorous population of the United States, or indeed that they are more than a sterile pseudo-philosophical exercise. A much more serious threat to present practice is not the crazed antivivisectionists but, rather, some sensible criticisms from sensible people. Senator Robert Dole, for one, has introduced legislation that, while respecting the absolute freedom of researchers to design scientific protocols and to make their own judgements on the necessity of using animals, would clamp down on some of the more serious persisting abuses in the care of laboratory animals and their use. The bill would require the use of analgesics, tranquillizers and anaesthetics except when scientifically impossible, would forbid the use of the same animal in more than one major operative procedure and would require either proper postoperative care or prompt euthanasia. Enforcement would be left largely to the animal care committees, which would have to certify in regular reports that experimental procedures involving animals followed these requirements. Significantly, the bill does not limit the freedom of scientists to proceed with such experiments; it does, sensibly, require that investigators "consider" alternatives and consult with a veterinarian in planning procedures involving unanaesthetized animals.

There is hardly anything subversive about these proposals. (Nor is there anything subversive in demands of animal welfare activists, such as Mrs Christine Stevens of the Animal Welfare Institute, that inspections now required by federal law should actually be carried out to ensure that laboratory animal care meets the published guidelines — which means basic things like clean cages, proper food and proper ventilation.) The US Department of Agriculture, which is charged with carrying out the inspections, has never been given the funds to discharge its responsibilities under the law. Yet such inspections as it is able to carry out reveal continued problems in these basic necessities for humane care. NIH would do well to face up to these issues, rather than issuing pronouncements full of sound and fury that signify nothing. □

Star wars

Pre-election Congress turning sour

Washington

THE militarization of space may yet become a significant issue in November's presidential election in the United States. For the first time, Mr Walter Mondale, the Democratic front-runner, last week promised to order a freeze on space weapons if he is elected President. In Congress, meanwhile, even the Republican Senate is alarmed about President Reagan's desire to press ahead with deployment of anti-satellite weapons and research on a "star wars" defence against nuclear missiles. Dr George Keyworth, the President's science adviser, was given a frosty reception when he told the Senate Foreign Relations Committee that he opposed negotiating a ban on space weapons lest it blocked the star wars programme.

Congressional anxiety can only have been heightened by the publication of a report by its own research arm, the Office of Technology Assessment (OTA), concluding that the prospect of creating a near-perfect defence is "so remote that it should not serve as the basis of public expectation or national policy". Although detailed criticisms of the star wars plan have been published before — most recently by the Union of Concerned Scientists — the new OTA study is the first to be published by a neutral body with full access to classified information. Its pessimistic conclusions are likely to have considerable influence on the mood of Congress.

The report, prepared by Dr Ashton Carter of the Massachusetts Institute of Technology, covers largely familiar territory by drawing attention to the difficulties of propagating directed energy weapons with the range and accuracy to knock out a large-scale nuclear attack, and pointing out that for every defence concept proposed so far, simpler and usually cheaper countermeasures have already been identified. One of the most forthright parts of the report, however, points to four common "misapprehensions" about the prospect for a perfect defence. They are:

- Confusing the successful development of single devices — lasers, mirrors, aiming mechanisms and the like — with the creation of a successful system. Multiplying by even a million some directed energy weapon will not necessarily give a perfect defence.
- Equating star wars with past technological challenges, such as the Moon landing or the Manhattan Project. The report notes a "vital difference" between working around constraints imposed by nature and competing, in the case of star wars, with a hostile intelligence bent on sabotaging the effort.
- Hoping for a technological development that will dispel all difficulties; when perfection of the X-ray laser for example, might turn out to benefit offence more than defence.
- Expecting to know with reasonable confidence how a complex defensive system would perform in reality. Unlike defences used in previous wars, this one would have no chance to learn or adapt.

OTA concedes that it might make sense to construct a star wars system even if it is less than perfect, in order, for example, to protect missile silos or simply to protect as many lives as possible. But it complains that, despite the fanfare with which President Reagan's star wars proposal was announced, its precise aims remain unclear. For that reason, explicit standards against which its technical feasibility can be measured have proved elusive.

Peter David

Genetic engineering

Genentech claims factor VIII

ONE of genetic engineering's glittering prizes was claimed last week by Genentech Inc., the San Francisco-based biotechnology company, working in collaboration with the Haemophilia Centre at the Royal Free Hospital Medical School, London, and Speywood Laboratories in Wales. The human blood protein known as factor VIII, which is lacking in haemophiliacs and essential for their treatment, has now been cloned and expressed in biologically active form in a mammalian cell culture. The availability of a completely sequenced cDNA clone for factor VIII, the largest protein ever produced through recombinant DNA technology, is an essential first step towards a source of the factor that is independent of supplies of human blood plasma and so not susceptible to contamination by viruses.

Most factor VIII used in Britain is prepared from concentrates that may contain the pooled plasma of up to 20,000 blood donors. All such concentrates are infected with hepatitis viruses and many haemophiliacs suffer from liver dysfunction caused by non-A and non-B hepatitis. Existing alternative production methods may lead to a lower risk of contamination but have their own disadvantages. Haemophiliacs are also a high risk group for acquired immune deficiency syndrome (AIDS), almost certainly because an AIDS virus can contaminate factor VIII produced from plasma. It has been clear for the past three years that any replacement of comparable efficacy would be assured of a huge market.

The Haemophilia Centre in London developed a method of obtaining factor VIII in high purity using monoclonal antibodies to the protein and established the gross structure. At Genentech, a genetic probe derived from a factor VIII subunit was used to test genomic libraries until a clone was identified that matched one possible predicted DNA sequence. The factor VIII gene turns out, as expected, to be located on the X chromosome. Eventually a clone of the complete gene was obtained (190 kilobases long) and a cDNA clone of factor VIII produced. After transfection of the clone into a mammalian cell line (Genentech will not say what type), human factor VIII was detected in the culture medium and measured by a purified enzyme system. The activity of the material is quenched by monoclonal antibodies to human factor VIII — further evidence that factor VIII is being produced.

Genentech is at pains to point out that there are several years' more work ahead before factor VIII will be produced by genetic engineering on a commercial scale, though it hopes to produce enough factor VIII for clinical trials of the product (probably around 100 mg) within 2 years. The aim now will be to obtain a cell line able to sustain high output of the protein and which can also be cultured on a relatively large scale. Last week's announcement preceded any publication of the genetic discoveries in a refereed scientific journal, but both Genentech and the team at the Haemophilia Centre in London say that papers are planned.

Naturally, nothing is as yet known of the economics of factor VIII production by genetic manipulation. There are, however, about 20,000 users of factor VIII in the United States, and as many again in Western Europe. Supplying known needs would be worth sales of at least $100 million a year. Knowing how to manipulate large molecules such as the components of the blood-clotting system will be worth much more to the company which first succeeds in making such materials.

The announcement last week will be a blow for several other biotechnology companies working on factor VIII. Previously, only parts of the gene had been sequenced. Genentech says it intends to protect fully the processes it has developed by patents, and a spokewoman added ominously that the company had recently expanded its patent staff. But the usefulness of patents in protecting biotechnology processes generally is not yet established, and one disgruntled competitor summed up his feelings by saying "better late than never".

Tim Beardsley

Cancer research

Direct-mail medicine criticized

Washington

A NEW organization created to raise funds in the United States for studies of diet, nutrition and cancer is coming under fire for spending only a small proportion of the millions collected through direct-mail solicitations on actual research. The American Institute for Cancer Research, founded in 1981 by two professional fund-raisers in the Washington area, took in some $3.5 million during its first fiscal year ending September 1983, while awarding only $400,000 in research grants.

According to a financial disclosure filed with New York State on 16 April this year, $600,000 was spent on public education and $2.4 million on fund-raising. And a report issued last month by the Council of Better Business Bureaus (BBB), an independent group that monitors charities and investigates consumer complaints, revealed that $893,000 of the institute's fund-raising expenditure went to the firm owned by the institute's founders, Jerry Watson and Chat Hughey.

The BBB report concluded that the institute failed to meet its standards for charitable solicitations, which require that at least half of the funds raised be applied to the programmes described in the organization's public appeals. The BBB standards also require that the organization should have an independent governing body.

Since the end of the fiscal year, the institute has made some changes to correct these deficiencies, notably by amending its by-laws to restrict the founders' rights to appoint or remove directors of the institute.

A spokeswoman for the institute said that New York State, which has the strictest disclosure laws in the country, included under "fund-raising" close to a million dollars in expenditures that the institute considers to be "public education" activities, such as the printing of newsletters. According to an audit commissioned by the institute itself, only 37 per cent of its receipts went on fund raising. The New York rules consider an activity such as mailing a newsletter to be fund-raising if it is combined with an appeal for funds and if fund-raising is the "primary purpose".

The direct-mail pieces that the institute is continuing to send out, however, have raised the hackles of many scientists, the American Cancer Society and, according to a report in the *Los Angeles Times*, the state attorney general's office in California. More than 11 million copies of a "Census on Diet and Cancer" were sent out, which combines on a single page questions such as "what vitamin supplements do you take?", "do you eat red meat at least three times a week?" and "have you ever had cancer?" with a "contribution reply" that offers the opportunity to make a tax-deductible contribution. Respondents are asked to tick a box that reads: "I agree! It's absolutely essential to do extensive research in the area of diet and cancer — especially now when it's estimated that 50 per cent of all cancer is diet-related."

The institute's spring 1984 newsletter quotes letters from enthusiastic donors, including such comments as "unfortunately medical schools appear to completely overlook (*sic*) the importance of proper nutrition in maintaining good health" and "I firmly believe . . . that many illnesses can be prevented, halted, and even cured by proper diet".

Dr Colin Campbell, a Cornell University biochemistry professor who served on the National Academy of Sciences panel on diet and cancer, was named senior scientific adviser to the institute last December. He acknowledges that the "census" continues to make him "uncomfortable" and that it is of virtually no scientific value. He said he had told the institute staff that it was unacceptable but had rewritten the questions to make them "neutral" at least. He said he had also rewritten a cover letter to make it less "scary" and more positive in tone, and the institute's fund-raisers had agreed to try it, although he said they had told him they were not sure it would work. The original letter had said "Cancer can strike you, your parents, your brothers and sisters — anyone".

Campbell said he had instituted a new process for reviewing grant applications modelled on that used by the National Institutes of Health study sections, and was taking steps to advertise the availability of grants. Until now, the availability of the funds had been spread only by word of mouth. Although the institute does not release the names of its seven-member review panel, a list obtained by *Nature* confirmed Campbell's assurances that they are all reputable research scientists.

Campbell said he felt it unfair to judge the institute harshly on its performance during its first year of operation. The spokeswoman said the institute hopes to disburse 20 per cent of its funds in research grants this year. And she cited as an example of the institute's activity in public education the mailing of 20,000 copies of the National Academy report to physicians and the distribution of one million cards on breast self-examination for women.

In addition to the $400,000 in grants made last year, a further $700,000 had been committed and is being paid out, according to Campbell. Campbell's new review panel met in March and approved a further nine research proposals. The largest recipients of the awards made last year were the Linus Pauling Institute ($70,000), the American Health Foundation ($50,000) and Theodore Krontiris of Tufts University ($50,000). Three members of the scientific panel, including Campbell, have also received grants. **Stephen Budiansky**

NIH to bolt the stable door

Washington

A NEW study by the National Institutes of Health (NIH) has recommended tighter procedures at their clinical research centres to prevent a recurrence of the Darsee affair. Dr John Darsee, who had been widely regarded as a brilliant young medical researcher at both Emory University and later at Harvard University, was found to have repeatedly fabricated research results.

The NIH study of Darsee's activities at the Emory General Clinical Research Center, one of 75 such centres supported by NIH, concluded that trainees should be supervised more closely and that co-authors of scientific publications should be required to accept responsibility, in writing, for the papers.

The study offered no recommendations specifically for Emory, noting that the university had taken action on its own to prevent a repetition following its internal investigation of the Darsee affair. A major weakness cited by the NIH investigation was the absence of procedures at Emory at the time to detect Darsee's unauthorized inclusion of faculty members as co-authors on his many publications and abstracts — and the failure of even witting co-authors to detect anything suspicious about Darsee's research results. The investigators were told that although the chairman of the department of medicine at Emory did routinely review all manuscripts produced by the department, his review was "primarily intended to insure that supporting grants and services are acknowledged correctly".

The recommendation for closer supervision of trainees arose from the investigators' discovery that the Emory faculty members with whom Darsee collaborated each assumed that another faculty member was supervising Darsee's clinical studies. The NIH study recommends the naming for each young investigator of a single supervisor who would be expected to review regularly the trainee's clinical studies, including raw data.

The NIH investigation was conducted by Drs Evelyn Hess (University of Cincinnati), Darryl DeVivo (Columbia/Presbyterian Medical Center) and James Freston (University of Connecticut). Its recommendations are now being considered by the NIH Division of Research Resources, which administers the clinical research centres.

Stephen Budiansky

Agent Orange

Veterans' case comes to court

Washington

THE complex saga of Agent Orange, 11 million gallons of which were sprayed on Vietnam between 1962 and 1971, will finally come to trial before a federal court in Brooklyn, New York, on 7 May. A jury will have to decide whether the chronic health problems of nine plaintiffs, including five veterans and three children born with catastrophic birth defects, were caused by exposure to dioxin contaminant tetrachlorodibenzodioxin (TCDD) found in the herbicide. A huge amount of money is at stake; the plaintiffs represent the entire class of veterans and families who attribute medical problems to exposure to Agent Orange. Damages awarded against the companies concerned could amount to thousands of millions of dollars.

Although the class action is directed principally against seven manufacturers of Agent Orange, the federal government is expected to be in court too — very much against its will. The manufacturers, claiming that the federal government knew about the possible health effects of the defoliant when it used it, have filed third-party complaints that could force the government to share in any liability. The courts have so far rejected the government's claim that it cannot be sued for injuries received during military service.

The question of who knew what about Agent Orange, and when, will be a central issue in the trial. One of the allegations made against the manufacturers is that one of them, the Dow Chemical Company, was worried about the toxicity of dioxin as early as 1965, but tried to hide its concern from the federal government. Dow vigorously denies the charge. It says its worries in 1965 concerned the exposure of its employees to large concentrations of TCDD during manufacture, not use of the finished product which contained vastly lower concentrations. It also maintains that the Department of Defense knew at least as much as the manufacturers about the components of Agent Orange and the toxicity of TCDD.

But Dow and its co-defendants intend to base their defence on scientific arguments. They maintain that not enough research has yet been done to conclude whether the health of Vietnam veterans and their families differs significantly from that of the rest of the population. The evidence that is available, they argue, suggests that Agent Orange could not have caused the health problems ascribed to it.

Information about the effect of TCDD on humans is indeed sparse, although Congress has occasionally rapped the American Medical Association over the knuckles for saying so. Most of the data available have come from studies of industrial accidents, where victims are typically subjected to high exposures for short periods, not to low exposures over a long period as in the case of troops in Vietnam. Even so, the only TCDD-related disorder about which there is no argument is chloracne, the skin disease.

Preliminary studies of the impact of Agent Orange have so far failed to establish a clear link between the herbicide and the many disorders — including birth defects, lymphoma, neuromuscular weakness and liver enlargement — for which it has been blamed by veterans. The scientific evidence has been further muddied by the findings of the Air Force's own epidemiological study of the personnel involved in the "Ranch Hand" operation, as the herbicide-spraying operation was called. The Air Force reported in March that there was little evidence that the Ranch Handers had suffered as a result of contact with Agent Orange. None had soft-tissue sarcomas or chloracne, although the incidence of certain birth defects, skin cancer and liver disorders was high.

Like many other studies of Agent Orange, the Ranch Hand results have been hailed as a victory by both sides in the dispute. The Dow Chemical Company points out that the skin cancers could have been caused by exposure to sunlight; the veterans' lawyers said the study showed that science was beginning to "catch up" with what the veterans already knew — that Agent Orange damaged their health.

The Ranch Hand study, however, is only one of more than 60 federal studies of the effect of dioxin being conducted at a cost of some $100 million a year. Because the number of Ranch Hand personnel was small, the study was not expected to be sensitive to rare illnesses, a point made forcefully by a National Academy of Sciences review of the Ranch Hand protocol in 1980. Meanwhile, a major research programme, including a study of identical twins of whom one brother served in Vietnam, is under way within the Veterans Administration.

At congressional insistence, however, the Veterans Administration has had to transfer responsibility for a major epidemiological study of veterans to the Centers for Disease Control (CDC) in Atlanta. The Veterans Administration is distrusted by veterans and has been accused repeatedly of reacting slowly and insensitively to veterans' anxieties about their health. The $70 million epidemiological study will be the biggest ever conducted by CDC. Some 30,000 veterans will receive a detailed health interview and another 10,000 will be given extensive two- or three-day physical examinations in what CDC hope will become the definitive analysis of the Agent Orange question. But the results will not start to emerge for at least seven years, too late to help the Brooklyn jurors in May. **Peter David**

Chinese nuclear industry

Advance on broad front

DISCUSSIONS on a possible $20,000 million deal in nuclear technology were a major feature of President Reagan's visit to China last month. Coincidentally, the President's visit came shortly after the second national congress of the Chinese Nuclear Society, which reviewed the current state of Chinese nuclear technology and explored ways and means of "uniting and organizing" large numbers of scientists to meet the challenge of the nuclear age.

Addressing the inaugural session of the congress, Defence Minister Zhang Ai-Ping noted that although China has built a "fairly comprehensive" nuclear expertise over the past 20 years, and has started construction work on its first nuclear power station, at Taishan, the application of nuclear technology to the economy has been neglected. Satisfying the needs of "economic construction and improving people's standard of living" must, he said, be a priority for the nuclear industry, although it must also "ensure the satisfaction of military needs".

In fact, considerable progress has already been made on the applications of nuclear technology. More than 1,700 nuclear radiation instruments are now used in the textile, printing, paper-making, plastics and petrochemical industries. Between four and five thousand oil wells have been logged by radioactive methods, radiation sand-gauges have been installed in 12 hydrometric stations along the Yellow River to monitor sand content of the water and tracer techniques used for safety surveys of reservoirs.

Future plans include two more nuclear power stations (one in Zhejiang Province and one in the Shenzhen Special Economic Zone) and two 450-MW combined heat and power units for the Jinshang petrochemical works in Shanghai. Feasibility studies are in progress for urban central heating systems. According to Jiang Xinxiong, the Minister of the Nuclear Industry, China plans to be self-sufficient in nuclear fuel by the end of the century, while experimental work on fast breeder reactors and controlled thermonuclear fusion is "making progress".

The main problem, according to Defence Minister Zhang, is, however, safety, and, at the same time, popularization of knowledge about the peaceful use of nuclear energy in order to dispel "misgivings and apprehensions". The Chinese nuclear industry, Zhang said, is quite capable of handling fuel reprocessing, but should go on improving facilities and techniques in this field. Some experts believe that it could also undertake reprocessing on a commercial basis for other countries. **Vera Rich**

Australian technology

Jones and Hawke make hay

Canberra

THE restructuring of Australian industry in the face of rapid technological change has become a central policy theme of the year-old Labor Government, due in large measure to the advocacy of the Minister for Science and Technology, Mr Barry Jones. The Prime Minister, Mr R.J. (Bob) Hawke, has set up a committee of federal ministers to coordinate policies relating to the many deep structural changes necessary. As well as Mr Jones, the committee includes the Ministers for Trade, Education and Youth Affairs, Industrial Relations and Defence Support, under the chairmanship of Senator John Button, Minister for Industry and Commerce.

On 13 April, Mr Jones released a draft national technology strategy to stimulate Australia-wide discussion of structural problems. His paper, a summary of deliberations of the National Technology Conference last September, contains several proposals crucial to the transformation of Australian productivity. It is particularly concerned with the minuscule investment in research and development by private industrial companies. According to the figures made available, research and development in Australia in 1981–82 hit a low of 1 per cent of gross national product (GNP), with the business sector's contribution sinking to 0.2 per cent. In 1968–69, private research and development was 0.5 per cent of GNP in a national total of 1.3 per cent.

The paper now sets a target of 1.5 per cent of GNP overall for 1990, with the business share amounting to 0.5 per cent and rising to 1.0 per cent by 1995 to equal the government's projected contribution.

Much of the blame for Australia's dismal export performance in technology was attributed to the country's attitude to education as an investment. The Jones strategy now urges that the proportion of Australian children completing secondary school be increased from 30 per cent in 1982 to 40 per cent in 1985 and 50 per cent by 1995. Alarmingly, the number of tertiary students between 17 and 22 years old actually fell, as a proportion of the population of the same age, from 11.5 per cent in 1975 to 10.5 per cent in 1982. The strategy paper's recommended figures for 1985 and 1995 are 12 per cent and 20 per cent respectively.

Many of the recommendations in this paper are politically sensitive, including the proposal for a review of union attitudes to adult apprenticeships, the effect on training of minimum wages for juniors and the previously sacrosanct doctrine of "comparative wage justice" in which "equal work" attracts equal pay regardless of the industry or its capacity to pay. One per cent of the workforce should be retrained each year, a portable superannuation scheme instituted to promote mobility between industry, universities and government, and industrial protection gradually reduced.

In a society arthritic with structural rigidity, with 50 per cent of the workforce unionized and a cadre of industrial managers not yet weaned from protectionism, the issues canvassed are strong meat. The prosecution of the policy implied will depend on Mr Hawke's skill at maintaining consensus.

An admirer of South-East Asian industrial development and a recent convert to economic planning in the style of the Japanese Ministry of International Trade and Industry (MITI), he claims that previous governments have not been interested in formulating coherent science and technology policies. **Jeffrey Sellar**

European pharmaceuticals

Free trade makes trouble

A BRITISH Medical Association (BMA) campaign aimed at preventing patients from being dispensed unlicensed drugs has suffered a temporary setback. The newly-formed Association of Pharmaceutical Importers claims that BMA's advice to physicians last week to stamp prescriptions "UK-licensed products only" contravenes the Treaty of Rome.

BMA's general medical services committee had become concerned by the growing number of reports of adverse reactions to imported drugs that are dispensed in the same ways as UK brands but which have not been licensed specifically for the UK market. Imported products bearing the same name as home lines may include differently coloured tab-

Radioactive atolls

Bikini Islanders sue United States

Washington

THE inhabitants of Bikini Island, which was used for 23 above-ground nuclear tests between 1946 and 1958, are suing the US Government in an attempt to force a clean-up of their homeland. The 167 residents of the island left at the request of the US military and have since been shifted several times to different nearby islands, living largely on food and supplies provided by the US Government.

A study commissioned by Congress reported last fall that a clean-up could be carried out at a cost of $90–120 million.



The most feasible approach, according to the study team headed by Harvard professor emeritus Henry Kohn, would be the removal of a million cubic metres of contaminated topsoil. Without such drastic measures, the island would not be habitable for 100 years if local foods were to be consumed.

The urgency of the suit filed this week, according to the islanders' attorney Jonathan Weisgall, is that the Compact of Free Association approved by the Marshall Islanders (of which Bikini is a part) and now pending before Congress would terminate all claims arising out of the nuclear testing, and would provide no funds for cleaning up Bikini. The Bikini Islanders would, however, receive $75 million in compensation over the next 15 years on top of the $23 million trust fund already set-aside by Congress. This fund is used to pay medical and other expenses and a monthly stipend to the 1,100 islanders and their descendants.

Eniwetok, an island near Bikini also used for nuclear testing in the 1950s, was cleaned up by the United States in the 1970s at a cost of $105 million.

The Bikini atoll had been resettled after the Atomic Energy Commission (AEC) reported in 1967 that radiation had diminished to a safe level. In 1975, however, an initial ground radiological survey raised doubts about AEC's earlier conclusion; and a full survey in 1978, which included measurements of body-burdens of radiation in the 139 Bikini Islanders who had returned, found that they were receiving exposures of 0.27 to 1.18 rem per year, largely from eating locally-grown food that had taken up radioactive caesium-137 from the soil. The federal standard for the general public is 0.5 rem per year; the occupational limit is 5 rem per year. Those who had resettled were removed once again, and the island has since remained uninhabited.

As well as taking legal action, the islanders are appealing to Congress for action on a clean-up; the House Interior Committee has recommended an initial $10 million authorization to begin the work.

Stephen Budiansky

lets and dosages, differently worded instructions (sometimes not in English) and may even be of different formulation or make-up. Its recommendation to physicians was intended to counter this trend.

BMA's advice is heartily endorsed by the Association of British Pharmaceutical Industry. British manufacturers estimate they lost £75 million last year to "parallel imports", bought from their overseas divisions and sold in Britain considerably below the standard British price. The price differentials are a consequence of the varied methods for regulating pharmaceutical companies in different countries. In Britain, overall profits are limited under the Pharmaceutical Prices Regulation Scheme, which specifies a target profit band for each company, based on capital employed, and also controls advertising expenditure. In other countries, prices or profits may be controlled individually for different products.

The European Court of Justice ruled in 1976 that parallel importing should be facilitated and that restrictions would be in breach of freedom of trade, but in 1980 an attempt by the commission to introduce a directive legitimizing parallel importing failed. The new pharmaceutical importers' association justifies its present imports by citing a British licences exemption order intended to apply "in circumstances in which small quantities of medicinal products are imported into the United Kingdom for the treatment of particular patients".

Even though advertising of products imported under this order is illegal, the Department of Health has been unwilling to take action against infringements, fearing it would be dragged through the European Court. A new statutory instrument that will permit advertising of parallel imports and comply with the European Court's ruling while retaining some controls is now being finalized by the Department of Health and will be laid before Parliament in a few weeks' time.

A spokesman for the parallel importers accepted that British patients had in the past been dispensed products other than those their physicians intended, but said the association will encourage its members to "put their houses in order". Recently, some of its members were obliged to give an undertaking in the High Court to stop passing off as the UK version an imported Glaxo product with deliberately counterfeit packaging.

At issue now is whether a voluntary professional association such as BMA can make recommendations to its own members without contravening European law. BMA says it took full legal advice before making its recommendations to physicians and is confident of their legality. The question now seems likely to be answered in court, and BMA has halted its campaign until the issue is resolved to its satisfaction.

Tim Beardsley

China in space

Triumph and martyrdom

CHINA'S space planners last month released details of the setbacks and disasters suffered during the nine-year effort to put into synchronous orbit an "experimental" communications satellite. The satellite was successfully launched on 8 April, and inserted into its final orbit at 125°E on 16 April.

The admission on Chinese radio that a number of "scientific and technical workers" had "laid down their precious lives" during the development of the satellite was clearly intended as part of the current drive against "anti-intellectual" prejudice still persisting from the Cultural Revolution.

The broadcast centred on two major examples of heroism. One, a lone martyr, Ma Jungyan, had been working on the possible effects of solar radiation on a satellite in orbit. As this was during the "chaotic time of the Gang of Four", Ma was obliged to carry out simulated experiments at the atomic energy research institute without even the "minimum safety protection". He was, said the commentator, fully aware of the danger but "knowing that there was a tiger in the mountains, he still headed towards them". As a result, he developed a serious radiation-induced lung condition, but during every remission continued to "work frantically".

The other incident was the explosion of a rocket on 28 January 1978, during tests on fuel flow. Seven of the research team, including the director of the test-pad, were seriously injured and several others suffered burns and ruptured eardrums. Nevertheless, at the urging of the injured director, a party meeting was held the same afternoon to determine the cause of the incident, and testing was back to normal three days later. A week after the incident, the director and "several other comrades" were back at work, still in bandages, and they returned to the hospital to complete their treatment only when the experiment was finished.

It is not clear whether there were any deaths in this incident; the commentator clearly implied, however, that there had been several fatalities on what he called the "battlefield" of the space programme, the programme of the rocketry division. Whether Ma Jungyan and his fellow martyrs will receive public recognition in the forthcoming exhibition on the Chinese space programme, which opens in Shanghai later this month, has not yet been revealed. But China seems bent on being open.

Vera Rich

Unesco

Looking for signs of change

FURTHER signs of the response of Unesco (the United Nations Educational, Scientific and Cultural Organization) to the discontent of some member states should become apparent next week, at the meeting of the organization's executive board arranged for 9–23 May. Those participating in the meeting expect that decisions about the budget for 1985, when the US notice of withdrawal will have expired, cannot be taken until the meeting of the executive board due in November. But next week's meeting should throw some light on Unesco's response to the British Government's for radical changes in the organization's way of working.

The agenda for next week's meeting concern's Unesco's science and technology sector only in uncontroversial ways — the executive board is expected to take on the nod the secretariat's proposals for the invitation lists to this November's meeting on information technology and that planned for next year on problems affecting Latin America and the Caribbean. Moreover, science and technology are relatively well protected in the secretariat's proposals for trimming $10 million from the two-year budget for 1984–85. Indeed, the grant to the International Council of Scientific Unions has actually been increased.

Fireworks, if there are any, will rather attend the general items on next week's agenda, especially that requiring a first response to the British letter from Mr Timothy Raison, Minister of State at the Overseas Development Administration, sent to the director-general of Unesco in March. That letter asked that Unesco should reorder its priorities and improve the management of its affairs. At a later meeting in London, the director-general was told that continued British membership "could not be justified" unless there were signs of change at Unesco.

By all accounts, few expect that there will be much that is tangible to report from this first meeting of the executive board since the letter was sent, although the British will at least be looking for some mechanism by which their proposals can be studied seriously. But the responses of both the Unesco secretariat and the delegates of other member states are likely also to influence the spirit in which the British Government carries out its promised — or threatened — review of the benefits of membership.

Mr Ray Beverton, who succeeded Sir John Kendrew on 1 April as chairman of the science and technology subcommittee of the British National Committee on Unesco, said earlier this week that his group had not yet begun work in earnest on the task, but that a meeting had been arranged for May. □

Animal welfare

UK compromise and coercion

THE British Toxicology Society has thrown its professional weight behind the campaign by British animal welfare groups to end the LD_{50} test of acute toxicity. A special report by a working group of the society concludes that accurately determined LD_{50} values are rarely justified, and proposes an alternative test which it considers more humane while still providing essential safety data for product labelling and classification.

The LD_{50} test has long been a main target of animal welfare campaigners. New guidelines published in 1981 by the Organization for Economic Cooperation and Development (OECD) lowered the number of animals needed for the test, which establishes the quantity of a substance needed to kill 50 per cent of a group of test animals. Even with the new guidelines, however, 30 animals would typically be used for each LD_{50} determination.

The alternative proposed by the British Toxicology Society classifies substances into broad bands of toxicity based on their observed effects on animals at pre-set dosages. If survival at one level is more than 90 per cent, with no evident sign of toxicity, dosage is increased by a factor of 10; if it is less than 90 per cent, dosage is decreased by the same factor. This procedure continues until the pre-set level is found that causes evident toxicity but allows more than 90 per cent survival, which specifies the classification. With 10 animals per group and 3 classes of toxicity (as in the current EEC chemical regulations), on average between 10 and 20 animals would be used.

The Home Office in London is working on new proposals for animal welfare legislation, and is keenly aware of public pressure to reduce the number of animals used in routine testing. Mr David Mellor, Under-Secretary of State at the Home Office, is known to be sympathetic to the view that the continued widespread use of LD_{50} should not be necessary. The Toxicology Society hopes its acute toxicity test procedure will gain peer acceptance and become internationally recognized.

The claimed advantages, apart from compatibility with existing European systems, include the fact that (unlike LD_{50}) signs of toxicity are included in the assessment, while severely affected animals can be humanely killed without affecting the outcome of the study. The new test is also likely to be less variable between laboratories than LD_{50}. The long-term aim of the society is to force a European or a UK initiative in OECD aimed at establishing new guidelines. An existing EEC directive on dangerous substances that seems to imply use of the LD_{50} test would probably need amending.

Meanwhile, the Home Office is still undecided on how to modify the controversial "pain clause" in the draft proposals on animal experiments that it published last year. It is being pressed by the British Veterinary Association and others to extend the existing ban on experiments that cause severe and enduring pain to those causing severe pain or distress. The association, together with the Fund for the Replacement of Animals in Medical Experiments (FRAME), is also pressing for a linkage between the amount of pain or distress that may be allowed in an experiment and the potential benefits. Home Office officials are now thought ready to accept the principle of such a linkage.

Civil disobedience and violent protest against animal experiments are likely to be stepped up over the coming months in Britain. A coalition of hardline antivivisection organizations linked under the banner of "Mobilisation for Laboratory Animals" is planning a week of protests that will culminate in a national march on 12 May. The demands include a ban on the LD_{50} test and the Draize eye test, as well as cosmetics testing and all behavioural, psychological and military experiments. Last weekend, a security guard was injured when protesters broke into testing laboratories owned by Imperial Chemical Industries Ltd. Some parties to the "mobilisation" coalition condone, if not actually organize, protests that entail physical violence, and more violent incidents seem likely. Later in the year, according to a spokesman, a vigil will be held outside every animal laboratory in Britain.

Tim Beardsley

Israeli–Egyptian relations

Cooperation in the doldrums

Rehovot

A LEADING Israeli physicist recently gave an "unofficial lecture" at a major Egyptian university. For political reasons, there was no advance notice of the lecture, organized by an Egyptian colleague whom the Israeli had met at a US conference. Word got around, however, and by the time the Israeli began to speak, the lecture room was crowded with young Egyptian researchers, most of whom stayed behind afterwards to discuss physics and to express the hope that full above-board links between Israeli and Egyptian scientists would soon be possible.

Although Israel and Egypt have been at peace for five years, there is at present only one place where scientists and scholars from the two nations can meet officially, the Israeli Academic Centre in Cairo headed by Professor Shimon Shamir of Tel Aviv University. The centre, sponsored principally by the Israel Academy of Sciences and Humanities, functions like the cultural centres of other nations, serving Israeli academics in Egypt for scholarly research, presenting lectures by these academics and giving Egyptian scholars access to Israeli research papers and books.

Such activities would be quite straightforward were it not for the fact that many in Egypt now regard all contact with Israel, no matter how innocent, as suspect.

Since the centre opened in May 1982, most of the lectures given there have been devoted to subjects of clear mutual interest. For example, Professor Sasson Somekh, an Israeli expert on Arabic literature and language, dwelt upon the fact that Hebrew and Arabic originate from the same linguistic family — and then dealt with problems of modernization in the same spirit. Similarly, Israeli musicologist Amnon Shiloah examined the influence of Arab music on Jewish music while Hebrew University Professor Hava Lazarus-Yafeh discussed the relationship between Halkha (Jewish religious law) and Sharia (Moslem religious law).

Sometimes, Israeli lecturers have reported on research carried out in Egypt itself. Dr George Kanazi, a senior lecturer in Arabic literature at Haifa University, told his Cairo audience about mediaeval Arabic manuscripts on the art of wine-drinking. Kanazi, himself a Christian Arab born in Nazareth, pointed out that even after the advent of Islam, which prohibits intoxicating beverages, laxity in the interpretation of Islamic law had made wine-drinking "a luxury that was sought and enjoyed".

Many of the Israeli researchers visiting the academic centre are interested in Islam and many of the Egyptians are similarly interested in Judaism.

Egyptian doctoral candidates have received information, for example, on "Elijah the prophet in Jewish and Moslem folklore" and "the author Aharon Megged as a representative of Israel's 1948 generation". Other PhD candidates have sought and obtained material for studies comparing Egyptian and Israeli approaches to education, social welfare and architecture.

The reactions of Israelis to these interactions, and to Egypt generally, are variable. Shamir says that "Israelis who come to Egypt looking for fanaticism, political rigidity and uncompromising hostility towards their country find these things; those who come looking for open-minded attitudes, warmth and a desire for peace find them also."

Shamir regrets that it is impossible to reach agreement just now for scientific cooperation between Israeli and Egyptian research centres but welcomes "the beginnings of a cultural interchange" at this centre.

Nechemia Meyers

Amersham International

Academic symbiosis flourishes

To sponsor a fellowship at the University of Cambridge, in a department (biochemistry) run by a knight (Sir Hans Kornberg) who is also master of a Cambridge college (Christ's), as Amersham International is about to do, may seem slightly odd for a company that thinks of itself as an adventurous creature newly emerged from the confining chrysalis of public ownership. But the Amersham Fellowship is a recognition by the company of the extent to which its growth, diversification and profit depend upon the progress (and demands) of academic research.

Dr J.C. Maynard, chief executive of the research products division — one of three into which the company is now organized — estimates that he has about 120 collaborations with academics, including sponsorship of several projects. That is largely why the company was able to market 130 new research products last year, many of them the result of an opportunist approach and many of them unrecognizable as products of what was, until 1981, the Radiochemical Centre.

The change of name followed a change of managing director and preceded the sale in 1982 of shares in the company by the British Government. (That move is reckoned to have cost the taxpayer dearly because of a gross underestimate of investor demand for the shares, which more than doubled in price after the sale.)

One striking change of business strategy has been that Amersham is taking a leaf out of the British dairy business's book. Just as the system of daily delivery of milk to British households is now being exploited to offer anything from meat to bread, so Amersham has decided to exploit its system for the speedy delivery of labile radiochemicals to market other products. The main thrust has been in the direction of molecular biologists. Since they all take deliveries of radiochemicals, why not offer them restriction enzymes as well?

Amersham seems to have made a considerable success out of just that, obtaining the enzymes from the Japanese market leader, Takara Shuzo. Perhaps five per cent of the sales of research products, which accounted for about a third of the company's £78 million turnover in 1983, is accounted for by restriction enzymes. But, as marketing manager Dr Brian Ellis is quick to say, Amersham faces much more competition in the sale of enzymes than in radiochemicals. Like all enzyme companies, Amersham would dearly like to have some unique enzymes on its list and would also like to offer reverse transcriptase, an essential enzyme for almost all molecular biolgists.

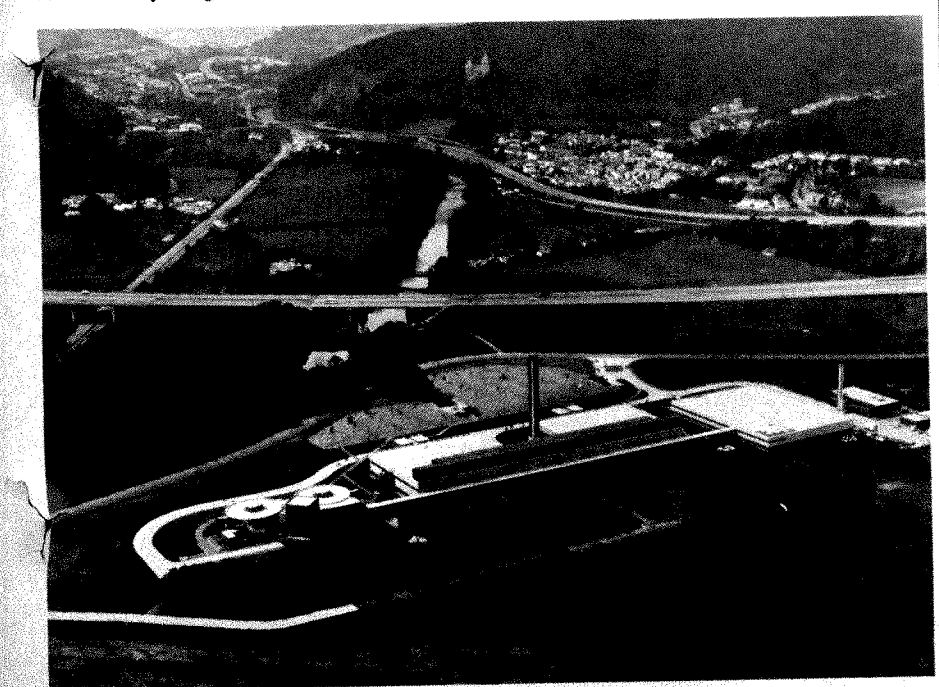
Other burgeoning sales to molecular biologists come from reagents for the cloning, packaging and sequencing of DNA. Most of the reagents are not radioactive, many are offered in convenience kits. (There is even an anti-DNA kit, not so much an anti-research product as an aid to diagnosing the presence of antibodies to DNA in clinical blood samples.) Like many other companies, Amersham is also looking hard at the possibility of supplying non-radioactive alternatives to the current DNA probes but so far none is of sufficient sensitivity, says product manager Mahomed Jassat.

More in the traditional line of the company, but typifying its new approach, are radioisotopically labelled ligands for receptors on the surfaces of cells. "It's a flavour of the month business", says Ellis. The trick is to be very quick off the mark to produce new ligands after their discovery and before they are displaced by something better. It can take Amersham as little as three months to launch a new product. Some may sell for not much longer than that, and to the tune of only a few thousand pounds, but still be worthwhile.

Financially as well as physically, Amersham has been growing since it went public in 1982. The half-year figures for 1983 show sales and profits more than 20 per cent up on 1982. More than 80 per cent of sales are overseas, business is booming in Japan and the company feels it is gaining on its arch-rival, New England Nuclear, in the United States. In physical terms, the staff of Amersham now tops 2,000, the administrative staff is to move into offices being built close to the Amersham site and research staff will soon start to occupy what was the Pollards Wood Research Station of the Institute of Cancer Research, a few miles away.

That move is part of the two-year plan under which expenditure on research and development will increase from 8 per cent of sales to 10 per cent. As long as price resistance does not hit sales, Amersham will soon be in better shape than ever to take advantage of the many ideas that seem to be flowing from its contacts in academic life.

Peter Newmark



Signs of growth — Amersham's new facilities at Cardiff.

Taxing problems

MANY readers of *Nature* who have been alarmed to learn that foreign visitors earning income in the United Kingdom will in future be taxed like residents (*Nature* 26 April, p.762) have nothing to fear. The proposed changes in tax relief for non-domiciled employees working in the United Kingdom for non-resident employers, now in committee stage in the British Parliament, do not affect those working as teachers or professors at a recognized educational establishment, or those who are undertaking research that is for the benefit of the general public.

People in these categories will continue to enjoy total freedom from taxation in the United Kingdom, provided that the visit is for less than two years. Under double taxation relief agreements, this exemption is reciprocal and so would apply also to British academics visiting foreign countries to teach or carry out research.

Those unfortunates who are not teaching, or whose research is for the benefit of private individuals or companies, will not in future benefit from the special reduction for short-term visitors to the United Kingdom, currently calculated at a deduction of 50 per cent on earnings. From 1987 the relief will be reduced to 25 per cent and from 1989 it will be withdrawn entirely. □

Labs worth visit

SIR — Your cheap sneer (*Nature* 15 March, p.24) that "many once adventurous laboratories have become places where ageing investigators brood on their pension rights" perhaps reflects the fact that your reporters are no longer visiting these laboratories. If they did, they would recognize the exciting and high calibre research which is being conducted against a background of continuing cuts in both staff and equipment. They might also recognize the high regard with which these laboratories are held by overseas scientists if we are to judge by the number of requests received from scientists in developed and developing nations alike to visit and work in these laboratories.

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Nuclear abstracts

SIR — M. A. Bray (*Nature* **307**, 206; 1984) had expressed concern about the large number of abstracts appearing in databases. J. R. Metcalfe (*Nature* **308**, 222; 1984) questioned whether conference abstracts should even be included in databases.

For the nuclear physics bibliographic databases maintained at the US National Nuclear Data Center (NNDC), secondary references such as meeting or conference abstracts and dissertation abstracts are routinely coded. In the bibliography to neutron-induced reactions (CINDA system), all references to a given research work (either experimental or theoretical) are blocked together in the database. Abstracts are entered with a "no book flag" so that they never appear in the semi-annual cumulative publication, unless the abstract constitutes the only reference in that block. However, all entries can be obtained in a computer retrieval. The same was true for the charged particle induced reaction bibliography (CPBIB) system.

For the nuclear structure bibliography (Nuclear Structure References system) which is published thrice annually, there is no blocking. However, the secondary references appear in a section separate from the primary journal listings. In support of Metcalfe's arguments in favour of inclusion, the interval between an initial abstract and a published paper can be from one to five years. This time factor is significant (1) to alert a potential researcher that another group is already working on the same problem which could possibly avoid duplication and wasted research funds and (2) to direct a potential user to the researcher for more details on the work prior to publication.

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They are not alone

SIR — Is the individual research scientist becoming an endangered species, as more and more disappear into the relative obscurity of "research teams"? An investigation of the pages of *Nature* suggests that this is indeed the case.

Since 1979, the number of "Letters to *Nature*" published has been in the region of 1,200 per year. However, throughout this period, there has been a gradual annual reduction in the number of single — and double — author papers coupled with an overall decrease in manuscripts with three named authors. This marked decline has been accompanied by a major increase in the number of multi-author papers.

A breakdown of the 1979 and 1982 quota of Letters to *Nature* clearly illustrates the change of distribution. During the four years, the decrease in letters with three authors or less was reflected by the steady rise in the average number of authors per letter, the 1982 figure standing at a mean of 3.17 authors per paper which represents a 14 per cent increase overall.

The observed shift towards multiple authorship in *Nature*, illustrated by the presence in 1980 of a 2½ page article with 27 named authors, poses the question: has scientific research become specialized to such an extent that individuals must now pool their expertise in order to successfully carry out a research project?

The most likely cause for the conspicuous movement away from single-authorship is, perhaps the "publish or perish" syndrome. Drastic funding cutbacks in the private and public sectors, worldwide recession and the threat of unemployment have all intensified the pressure on researchers to protect their positions. Being named on as many publications as possible, no matter how remote the connection with the original research work, is one way of proving one's competence.

ANDREW J. CRUMP
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Numbers of Letters to *Nature* with 1 to > 9 authors in 1979 and 1982.

	1	2	3	4	5	6	7	8	9+
1979	198	448	307	161	65	34	13	9	11
1982	134	360	265	170	83	67	28	17	12
Mean change per year (expressed as %)	−9	−7	−2	+5	+13	+35	+42	+39	+7

Homer's *vino*

SIR — Will someone in the Mediterranean please confirm or refute Wright and Cattley's chemical hypothesis about Homer's "wine-dark sea" (*Nature* **303**, 568; 1983). I tried with French wine using Copenhagen water that is alkaline enough to turn red cabbage blue, but all I got was a tasteless red fluid. Perhaps "wine dark" refers to the lack of turbidity in wine. Coastal and inland waters are usually turbid whereas the clarity of deep Mediterranean waters off rocky shores could be compared to the clarity of wine. If so, "wine clear sea" would be a better translation and close to the Dutch/Afrikaans (?) *wijnkleurige zee* quoted by Macnamara (*Nature* **307**, 590; 1984).

The use of οινοπα ποντον as a fixed Homeric metaphor without necessary reference to colour has an analogue in modern English. What is the skin colour of most of the *white* men you have seen recently?

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Exciting news

SIR — Your reviewer (*Nature* **307**, 765; 1984) suggests that some results may "titivate". It is hard to see how they could spruce up either themselves or your readers, although the results might well agreeably excite (titillate) at least the latter.

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Only correct

SIR — Two errors are becoming commoner in scientific writings.

(1) The word "only" is being shifted forward, away from the word it modifies. Note the differences among the following: "Only I saw the white shark" (no-one else was on deck). "I only saw the white shark" (I didn't actually catch it). And "I saw only the white shark" (there were no grey ones around).

(2) Within the past few years the word "within" has been supplanting the simpler "in", usually without cause. Rats live within cages, particles sediment within a limited space, trees within a given area grow and die. In such contexts, what is wrong with using "in"?

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The real thing

SIR — Your correspondence columns have recently contained proposals for various "opposites" to placebo. The German-speaking world has used *Verum*, "the true thing", happily for years as an antonym to placebo. I managed to use it in an English language journal (*J.R. statis. Soc.* A **146**, 386; 1983), although the editors insisted on printing it in italics.

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Benefits of measuring time

Further data from the eccentric binary pulsar reaffirm the value of observations extended over time. The reality of gravitational waves is confirmed, the masses of the stars remain a puzzle.

THE old adage that if you must make measurements, you had better use a clock for then you will have time on your side, has been verified again. Almost exactly five years ago, Professor J.H. Taylor, the radioastronomer then at the University of Massachusetts at Amherst, described the first measurements of what is affectionately known as his pulsar (*Nature* **277**, 437; 1979) that were accurate enough to constitute a proof that gravitational radiation is real. In fact, the object that Taylor has been observing closely for the best part of a decade is not an isolated pulsar, but a binary system — a neutron star and a companion star of roughly equal mass.

The good luck is that the two objects are tightly bound — the orbital period is 7 hours 45 minutes — and that the orbit is also markedly elliptical. And the consequence of that is that the precession of the orbit resulting from non-Newtonian gravitational forces is rapid — more than 4 degrees a year, compared with a mere 0.43 seconds of arc a year for the precessing of the perihelion of the planet Mercury in its orbit about the Sun. Gravitational waves enter because the orbital period (not in this connection to be confused with the rotational period of the pulsar of 0.059 seconds) is decreasing, which implies that the system is losing energy in a way that can be explained only by gravitational radiation.

Taylor, now at Princeton University, has now, with J.M. Weisberg of the same laboratory, brought the story of the pulsar 1913 + 16 up to date (Weisberg and Taylor, *Phys. Rev. Lett.* **52**, 1348; 1984). Observations with the 300-m Arecibo dish in Puerto Rico have been accumulating for the whole of a decade. Altogether, there are more than 3,300 separate measurements, each consisting of the integration of the 5,000 or so pulsar wave forms received in a 5 minute interval. The authors say that their binary system is "uniquely suitable" for testing the prediction of general relativity that the members of a binary system should "gradually spiral together as the system loses energy in the form of gravitational radiation", but many of their readers will be more impressed by the astonishing precision with which the orbit of the binary system can now be described.

Inevitably, the orbital period is the most accurately determined of all the quantities involved. The standard error of the period, in round numbers 27,907 seconds, is a mere 20 microseconds, comparable with the standard error of a single measurement of the time of arrival of the individual pulses in one of the elementary observational periods of 5 minutes. But with a terrestrial atomic clock accurate to 1 microsecond, should it not have been possible to do even better after 10 years of observation? With more than 1,000 orbits every year, 10 years of observations should after all give a periodic time accurate to within the standard error of the clock divided by 10,000, say a nanosecond or so. And are not the rotational periods of many pulsars known to an accuracy of better than 1 picosecond?

The snag is that a system is much more complicated than an ordinary bare pulsar. With both kinds of objects, of course, it is necessary to correct the times at which pulses are expected to arrive for the physical motion of the Earth in its orbit around the Sun (which can make a difference of more than 2,000 seconds for an object in the plane of the ecliptic). For a binary system such as Taylor's, it is necessary to extract from what is essentially a sequence of time-marks all the characteristics of the pulsar orbit. One practical difficulty with Taylor's pulsar is that its signal is weak, though reproducible, so that the usual trade-off between receiver bandwidth and time precision arises.

With the passage of five years, however, the precision with which quantities such as the eccentricity of the orbit (numerically, 0.617 and now known to 3 parts in a million) can be known has been improved by a factor of at least two. The most striking improvement has come in the estimate of the projection of the semi-major axis of the elliptical orbit on the line of sight (a factor of five), the rate at which the orbital period is decreasing (a factor of seven) and, most important, the rate of precession of the orbit (a factor of six). A decade from now, this distant orbit may be as well known as, say, the orbits of some of the satellites of other planets in the Solar System.

Conceivably, the precision of some of these quantities may be dramatically improved by a technique Dr A.G. Lyne of the Nuffield Radio Astronomy Laboratories of the University of Manchester at Jodrell Bank has been exploiting in the study of another binary system one of whose members is also a pulsar. By all accounts, it is possible to exploit the irregular pattern of ionization in the general direction of a pulsar which is part of a binary system to infer the position of the pulsar in its orbit from the scintillation of the pulsating radio signal. For practical purposes, the ionization pattern in the intervening space functions as a kind of scale against which the position of a pulsating star can be pinned down. No doubt Taylor will be anxious to see whether the space intervening between here and PSR1913 + 16 allows this to be done.

Even as things are, however, Weisberg and Taylor have a good deal to say. In 1982, Taylor and Weisberg (*Astrophys. J.* **253**, 908; 1982) were saying that the data accumulated by then ruled out all gravitational theories except general relativity and the Brans-Dicke theory. Now they say that only Einstein's theory will square with the observations.

Weisberg and Taylor also say that their data now reveal a delay in the signal from the pulsar in those parts of the orbit from which the line of sight passes through the gravitational field of the companion star. This is the phenomenon now frequently observed when the line of sight to a pulsar grazes the limb of the Sun. Weisberg and Taylor say that their data constitute the first observation of this effect outside the Solar System.

The other striking and newish development is that the masses of the two stars have now been pinned down much more precisely than has previously been possible. The essential difficulty is familiar — that the masses of celestial objects whose gravitational interactions are those of point masses cannot be derived from Newtonian mechanics. But for the binary pulsar, the relativistic corrections, the advance of the periastron for example, are necessarily the least certain. But these are precisely the properties of the system whose precision has been most strikingly improved in the past five years. And the outcome makes more remarkable what has seemed from the start a strange coincidence — the masses of the two stars are indistinguishable both from each other and from the Chandrasekhar limit for collapse to a neutron star. So is it just possible that each of the stars is a neutron star, but that only one of them is a pulsar? It may be possible to learn something about the nature of the companion star if the measurements become precise enough for mutual tides to show up, but on the face of things not even the passage of time will allow this intriguing question to be answered.

John Maddox

Acquired immune deficiency syndrome

Retroviruses linked with AIDS

from Robin Weiss

THE publication of four papers[1-4] in the 4 May issue of *Science* by R. C. Gallo and colleagues on a link between acquired immune deficiency syndrome (AIDS) and a retrovirus related to human T-cell leukaemia virus (HTLV) was preceded by much comment which has generated considerable confusion. The main causes of this sorry state of affairs are the intense interest in, and fear of, AIDS and the decision that the US Secretary of State for Health should herself announce 'the identification of the cause of AIDS' and hence the 'means of prevention'. Moreover, rivalry and lack of cooperation between different US governmental agencies — the Center for Disease Control, the National Institute of Allergy and Infectious Diseases and the National Cancer Institute (NCI) — led to uncoordinated press statements. These have exacerbated the already contentious matter of deciding precedence between L. Montagnier's group at the Institut Pasteur in France, which published first[5,6] but with skimpy data, and Gallo's group at NCI, which delayed submission until a thorough characterization of their virus and repeated isolations from different patients had been accomplished. Furthermore, the two groups are using three different names for what I believe will turn out to be the same virus.

Why look for a virus?

There no longer seems to be any doubt that AIDS is caused by an infectious agent. The view that it might instead result from repeated exposure to different human antigens, while plausible for homosexuals with multiple sexual partners and for haemophiliacs receiving factor VIII pooled from thousands of blood donors, cannot apply to AIDS developing after a single blood transfusion or in infants of affected mothers. What kind of infectious agent, then, might give rise to this disease? Again, the discovery of the transmission of AIDS by blood or by semi-purified blood products helped narrow the field because it is unlikely that a fungus or a bacterium (perhaps secreting an immunosuppressive toxin comparable to cyclosporin A) would be transmitted by such routes. In any case, no sooner had AIDS been categorized as a new, epidemic and usually fatal syndrome in 1981, than the search for its causative agent was directed mainly towards viruses.

AIDS patients, and those with extended lymphadenopathy syndrome (ELAS) which may be either a milder form or early phase of AIDS, are infected with many viruses. Cytomegalovirus, Epstein-Barr virus and hepatitis B virus are examples of ubiquitous persistent infections in the population at risk as well as many other individuals. They may appear in more virulent form in AIDS and ELAS patients but that is probably a result rather than a cause of the disease. In turn, they may play a part in exacerbating immune deficiency once it sets in, but these well known widespread viruses did not seem likely to be the initiating agent of AIDS. Instead the pointers were towards a new virus, or a new satellite agent which might increase the pathogenicity of a common virus, just as the delta agent causes hepatitis B to be more severe.

Montagnier's viruses (LAV and IDAV)

Last May, Montagnier and colleagues reported the transient production of cell-free reverse transcriptase activity and virus particles when leukocytes from an ELAS patient were co-cultivated with fresh T cells from normal subjects[5]. The virus showed no cross-reaction with the core antigens of HTLV-1, the virus that is frequently associated with human T-cell leukaemia, though there was slight cross-reaction with antigens on the membranes of HTLV-1-infected cells. Montagnier named the virus lymphadenopathy virus one (LAV-1). Scepticism of the French group's observations grew because other laboratories had difficulty in confirming them and because of a widely held opinion among electron microscopists that the particles depicted in the paper resembled an arenavirus more than a retrovirus.

At the Cold Spring Harbor Meeting on HTLVs last September, Montagnier presented further data, including electron micrographs of particles that resembled D-type retroviruses with truncated (cylindrical) cores and long stalks where budding of the virus from the plasma membrane of infected cells was incomplete[7]. The major core antigen, p25, of LAV-1 was found to be antigenically related to equine infectious anaemia virus (EIAV), a little-studied retrovirus that causes haemolytic anaemia and fever in horses. In its ultrastructure, EIAV is similar to LAV-1 as seen in the more recent electron micrographs[7,8]. At Cold Spring Harbor, Montagnier also described further isolates of retroviruses from two AIDS patients[7], one of them a haemophiliac. The virus from the haemophiliac and a virus isolated from his brother, who is also a haemophiliac but does not have AIDS, have recently been described in *Lancet* [6] and have been named immune deficiency associated viruses (IDAVs). IDAV is indistinguishable from LAV.

These viruses are promising candidates as aetiological agents of AIDS and ELAS because 60–75 per cent of AIDS patients have serum antibodies that react with the p25 antigen of LAV-1, and because they are cytotoxic *in vitro* to the OKT4$^+$ subgroup of T cells which contains the majority of helper T cells. To date, LAV-1, IDAV-1 and IDAV-2 have not been thoroughly characterized biochemically, and permanent cell lines producing the viruses are not yet available, although nearly a year has passed since the first report of LAV appeared in print[5].

Gallo's virus (HTLV-3)

The virus now described by Gallo *et al.* has been designated HTLV-3 because it is considered to be a new member of the HTLV family (now called lymphotropic, instead of leukaemia, viruses). Some 50 HTLV-3 isolates have been obtained from different AIDS and ELAS patients with the ingenious use of a T-cell leukaemia line (HT) that is highly permissive to HTLV-3 replication[1]. The infection of HT by HTLV-3 is easily detected by the formation of syncytia similar to those we described for HTLV-1 and HTLV-2 (ref. 9). The continuous production of high titres of HTLV-3 in HT allowed Gallo's group to characterize the viral proteins and prepare antibodies to them. Schüpback *et al.*[3] report a cross-reaction of HTLV-3 p24 with other HTLV strains, particularly HTLV-2. Genome analysis, yet to be published[10], indicates that HTLV-3 is significantly related to HTLV-1 and HTLV-2.

More than 80 per cent of ELAS and AIDS patients have serum antibodies that are related to specific HTLV-3 antigens, especially the envelope glycoprotein, gp41. A relatively high proportion of a small sample of homosexuals and drug addicts without AIDS also have HTLV-3 antibodies.

Are HTLV-3 and LAV/IDAV the same virus?

The NCI group place their AIDS virus firmly in the HTLV family whereas the French group have emphasized that their virus has antigens that cross-react with EIAV and that it lacks relationship to HTLV-1. Nonetheless, in my view the similarities between HTLV-3 and LAV are more remarkable than the discrepancies. Disregarding the original electron micrographs of Barré-Sinoussi *et al.*[5], the ultrastructure of both viruses is very similar (compare Fig. 2 of Vilmer *et al.*[6] with Fig. 1 of Gallo *et al.*[2]). While HTLV-1 and HTLV-2 endow OKT4$^+$ cells with indefinite growth potential, neither HTLV-3 nor LAV has this capacity for 'immortalization'. Both HTLV-3 and LAV can be passaged as free virus, whereas HTLV-1 is much less stable and unlikely to survive the process of factor VIII preparation — although we have reported cell-free transmission of HTLV-1 (ref. 11).

The apparent differences in immunological cross-reactions could be explained by the different tests and reagents used by

the two groups, particularly as HTLV-3 is more distant from HTLV-1 than HTLV-2 and most AIDS and ELAS patients lack antibodies recognizing p24 of HTLV-1. Given the relationship claimed between LAV and EIAV it will be interesting to ascertain whether the latter belongs to the HTLV family (which includes bovine leukosis virus). The relationship of HTLV-3 and LAV could be rapidly resolved by an exchange of reagents between the two laboratories.

A serological test devised in Essex's laboratory[12] (the 'HTLV-MA' assay) appears to correlate closely with AIDS and ELAS in homosexuals, haemophiliacs and other recipients of blood transfusions[12-14]. The HTLV-MA test is an immunofluorescence assay of the binding of serum to the surface of HTLV-1-infected cells, in particular to the gp61 envelope precursor of HTLV-1 that has been defined by Hattori *et al.*[15]. I have been frankly dubious about HTLV-MA as a specific test of HTLV-1 antibodies because of its lack of correlation with our more specific env antigen assays[9,16] and because faint fluorescence at 1 in 4 dilutions of serum were counted as positive. It now seems much more likely that the HTLV-MA weakly cross-reacts with HTLV-3 or LAV. No doubt Essex *et al.* will re-analyse their sera using a more appropriate antigen. Indeed, as mentioned earlier, a weak cross-reaction by live-cell immunofluorescence between sera of LAV-positive patients and HTLV-1-infected cells has been observed[5].

Immunodeficiency caused by other retroviruses

One of the reasons for suspecting that a retrovirus might be the cause of AIDS is that several animal viruses (including some strains of avian and feline leukaemia viruses) are known to cause various kinds of immunodeficiency, anaemia and aplasia. Indeed, it has not been lost on opportunistic retrovirologists that the US AIDS budget is a lucrative source of research funds. For years, the immunodeficiencies caused by some retroviruses were regarded as a nuisance because infected animals died of 'laboratory' infections before they could develop leukaemia. The focus of pathogenesis has curiously changed in recent months with an eruption of vintage viruses carrying new appellations such as FAIDS (feline AIDS) and SAIDS (simian AIDS).

The prototype D-type retrovirus, the Mason-Pfizer monkey virus (MPMV), was first isolated from a rhesus monkey in 1970 (ref. 17). Much effort was expended in attempts to demonstrate oncogenic properties of MPMV but as Fine and Schochetman's excellent 1978 review[18] relates, the only pathogenic effect of MPMV was runting and immunodeficiency. Recently, outbreaks of simian 'AIDS' in the New England and California regional primate centres have been closely associated with a new strain of D-type virus related to MPMV[19,20]; experimental inoculation of this virus into young monkeys induces the disease [20]. Simian AIDS differs from human AIDS in affecting a much wider spectrum of blood cells than OKT4$^+$ T cells; nonetheless the identification of a retrovirus with this disease is of considerable interest. The human AIDS viruses are not antigenically related to MPMV.

HTLV-1 in AIDS/ELAS patients

HTLV-1, until recently known as ATLV in Japan[21], is most closely associated with adult T-cell leukaemia–lymphoma[22], a malignancy of OKT4$^+$ T cells. This clearly influenced Gallo's search for a virus in AIDS patients as it seemed reasonable to surmise from a knowledge of animal retroviruses that the same virus that initiated clonal malignant transformation in one situation, or a variant, might destroy the same target cell population in another situation. Another hint came from the fact that opportunistic infections, as characterize AIDS, frequently accompany the appearance of HTLV-1-induced malignancy.

Last year, Gallo's laboratory reported the isolation of HTLV-1 and proviral DNA from homosexual AIDS and ELAS patients[23,24], and the same virus has been identified in Haitian infants with AIDS[25]. Using serological tests which do not cross-react with HTLV-3/LAV we have also found that about 5 per cent of ELAS patients in London have antibodies specific to HTLV-1 (ref. 26), a far higher incidence than in healthy homosexuals and random blood donors. The significance of HTLV-1 infection in subjects at risk of AIDS is not clear, but from its low prevalence it seems more likely to be a blood-borne infection reflecting the life style of the patients than an alternative aetiological agent of AIDS.

Prospects for controlling AIDS

If HTLV-3/LAV really is the cause of AIDS, and I find the evidence convincing, then several important developments should occur quickly. First, we need to know what proportion of patients at risk actually have evidence of infection. The preliminary report [4] that a substantial proportion of clinically normal homosexuals have HTLV-3 antibodies is worrying, if it is a forewarning of future illness; it is clear, however, that cofactors play an essential part in precipitating AIDS. Second, reliable ELISA screening tests for blood banks are urgently needed, and might have to be used as routinely as tests for hepatitis B virus. Third, there is the prospect of developing prophylactic immunization and perhaps intervention for infected individuals too. As Groopman pointed out in these columns last week[27], the economic and logistical impact of the identification of the cause of AIDS is formidable, and the seropositive individuals will face severe psychological and behavioural problems.

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100 years ago

In a crowded house on Tuesday last the Convocation of the University of Oxford passed the much-debated statute allowing women to enter for "certain of the honour examinations of the University." The statute has been opposed on very different grounds. The old Conservative Oxford School (fast becoming extinct among the resident teachers) of course objected to any change in favour of the higher education of women; with them went a portion for the High Church party, who look with disfavour on any proposal tending to bring women into intellectual competition with men. Others, again, opposed the statute on the ground that it was unfair to men, who have to keep certain terms and pass certain examinations within a specified time if they wish to enter for an honour school, whereas the statute allows women to enter for honours without the same preliminary examinations, and without restrictions as to time and residence. Others again feared an influx of young ladies into Oxford, as likely to destroy the manliness of the undergraduates and spoil the natural modesty of the lady students. To these arguments the success which the present halls for ladies in Oxford have met with is the best answer. Their presence has not revolutionised the University; they have not been a stumbling-block to discipline nor a rock of offence to the Church. The women's examinations were on the same subjects, and the papers were set by the same men, as in the men's honour examinations before this statute passed. Now the same papers will serve for both, trouble will be saved, and the women who obtain honours will win a certificate universally recognised throughout the country. From *Nature* **30**, 19, 1 May 1884.

Ocean ecology

Fixation of CO_2 in dark midwater zones of the ocean

from Paul Tett

IF the ocean began as a rich organic soup, it has since become so poor that many organisms have difficulty in getting enough energy and organic matter for growth and reproduction. The evolution of photoautotrophy must have been a breakthrough, allowing the light-driven uptake of simple inorganic compounds of carbon, nitrogen and phosphorus to supply the entire food needs of many planktonic plants. The illuminated superficial waters of the sea (the 'euphotic zone') are thus commonly seen as the main region of primary production, particularly by phytoplankton, whereas the deeper waters are seen as a zone of consumption in which heterotrophic animals and bacteria meet their energy demands by the oxidation of organic carbon supplied from the euphotic zone. On page 54 of this issue of *Nature*, Karl and his colleagues present evidence to suggest that bacteria in the midwater between the two zones can intercept the organic particles falling from the euphotic zone and, despite the absence of light, use the energy trapped in them to convert additional carbon dioxide into organic carbon (chemolithotrophy)[1].

As the earliest living organisms, prokaryotes probably exploited both inorganic and organic sources of energy and carbon until the development of the eukaryotes with their greatly improved efficiency of photosynthesis at high oxygen concentrations. That probably caused many of the remaining bacterial prokaryotes to specialize in heterotrophy — hence the traditional view of modern oceanic prokaryotes as degraders, consuming organic material originally produced by eukaryotic phytoplankton. Recent investigations of the smallest phytoplankton have, however, shown the importance of the prokaryotic cyanobacteria (once called 'blue-green algae') in oceanic primary production[2,3]. And incorporation of carbon dioxide by thermophilic bacteria, using energy obtained by oxidizing locally enriched sulphide, iron or manganese, has been suggested as a source of primary production in food chains found near volcanic vents in deep water[4,5]. From careful analyses of sedimenting material collected by free-floating traps at depths between 50 and 2,000 m in the eastern tropical Pacific Ocean south-west of Mexico, Karl *et al.* now add the suggestion that energy initially harnessed photoautotrophically by euphotic-zone phytoplankton for the incorporation of inorganic nitrogen (as well as carbon) can subsequently be chemolithotrophically used by midwater microorganisms to fix additional carbon dioxide[1].

The chemolithotrophic microorganisms involved are probably denitrifying bacteria using the ammonium produced by the action of heterotrophs on the organic material sinking down from the euphotic zone. By oxidizing this NH_4^+ to NO_2^- the bacteria can obtain sufficient energy to take up carbon and reduce it to organic compounds. This chemolithotrophic use of nitrification to provide energy for carbon incorporation is less efficient than photosynthesis which, under optimal conditions, yields 0.08 moles of organic carbon for each einstein of light. Karl *et al.* cite yields of 0.03–0.06 moles of carbon fixed for each mole of ammonium–nitrogen oxidized, and estimate that potential chemolithotrophic production by microbes in their study region requires a maximum ammonium flux of about 0.8 mmol per m^2 per day. This flux, if completely supplied from photoautotrophically produced material with a carbon to nitrogen ratio of 7:1, requires an annual photosynthetic production of about 30 g carbon per m^2, close to lower estimates of tropical oceanic primary production. The greatest estimated chemolithotrophic production, about 0.4 mg carbon per m^2 per day, is equivalent to an annual average of 146 mg carbon per m^2, clearly only a small proportion of phytoplankton production. The suggestion by Karl *et al.* that "the flux of energy out of the euphotic zone may greatly exceed that nominally contained in particulate organic detritus" thus overemphasizes the importance of midwater chemolithotrophy, but these microbial processes may well influence the organization of midwater food webs.

Chemosynthesis has, of course, been known for many years and may, in unusual regions, such as the Black Sea, rival photosynthetic production[6]. Oceanic chemolithotrophs have ample supplies of their carbon source; their problem is to find at near-normal oxygen concentrations a sufficient concentration of energy source. In the Black Sea, chemosynthesis makes use of the high concentration of reduced material accumulated in the deoxygenated waters. The thermophilic bacteria of the deep-sea volcanic vents probably exploit local enrichments of reduced compounds[4,5]. Perhaps the major novel contribution of Karl and co-workers is to document precisely a mechanism whereby chemolithotrophs can, through oxidizing ammonium associated with sinking particles, obtain sufficient energy for growth in the oligotrophic ocean.

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Igneous petrology

Turbulence in petrology — the behaviour of komatiites

from E.G. Nisbet

CONSIDER the business of stamp-collecting: an elegant and harmless occupation of considerable economic benefit to countless post-offices, a source of endless diversion and speculation to the philatelist. Consider now the effect on that peaceful hobby of the discovery, somewhere to the east of Laputa, of a whole new continent filled with hitherto unknown national post-offices. Imagine the excitement and confusion of innumerable collectors scurrying in for examples. Eventually order is restored, what purports to be a comprehensive philatelic history is written and the hobby settles down to its normal delightful ways — until someone points out that some of the new stamps, though not exactly forgeries, are subtle but highly misleading overprints. But it is not clear how to identify them. Is history to be rewritten? Confusion returns.

Igneous petrology may not quite be stamp-collecting but it, too, has its excitements, as when komatiites were discovered fifteen years ago. This sent many field workers scurrying to collect samples from which much has been learnt about the chemical properties of komatiites. Now Huppert *et al.*, on page 19 of this issue of *Nature*, cast doubt on some (but not all) of the current notions about the chemistry of komatiites from an examination of the physical properties of komatiite liquids[1]. More reassuringly, they also provide some explanation of how komatiite lava flows can produce extraordinary large plate-like ('spinifex') crystals of olivine, thereby resolving a conflict between experimental results and cooling models.

When discovered, komatiites were a

wholly new class of extrusive igneous rock, rich in MgO and generally of Archaean age (older than 2.5×10^9 yr). Apart from providing some of the best clues to the composition, temperature and workings of the mantle of the early Earth, they are also of considerable economic value for their large nickel sulphide deposits. Komatiite lavas must have been very hot (1,350–1,650°C) and have had very low viscosity (0.05–1 Pas). Moreover, moving bodies of komatiitic lava (such as magmas in conduits or flows) must have had high Reynolds numbers and thus generally have been turbulent and capable of eroding walls or floors to conduits[2]. From their detailed study, Huppert *et al.* show that komatiite lavas were typically turbulent, were accompanied by vigorous forced convection and that even for the narrowest fissures (0.3 m) their Reynolds number was probably far above the critical value for the onset of turbulence. Flow rates must have been very high: a 3-m-wide fissure would have had rates considerably greater than the largest eruptions in historic time.

In turbulent flow, mixing maintains a uniform temperature, heat transfer is much greater than in a laminar flow and, thus, the ground below the flow will heat up rapidly. Huppert *et al.* show that erosion by melting of this underlying ground would be rapid and significant. Assimilation of the melted ground would introduce up to 10 per cent contamination into the lava. Contamination would be of minor geochemical importance if the lava flowed over a ground made up of fresh subaerial komatiite flows; but if it flowed over hydrothermally altered flows, or over other rock types, or if it had arrived through a conduit lined with older rocks, the contamination could be of major significance. Fortunately for those making thermal models of the Archaean Earth, the MgO content of the lava (from which temperature estimates are principally derived) is not likely to have been changed much by contamination but the trace-element content could have been very substantially altered. Thus, radiogenic isotopes could be assimilated from an older country rock, leading to artefacts in dating. That may be the explanation for some anomalously old Sm–Nd dating of komatiites[3]. How reliable, then, is information from the incompatible trace elements, such as Ti, which has been used to construct geochemical models[4] of the source mantle? Huppert *et al.* point out that contamination is likely to vary greatly from place to place within a flow so that any set of flows which displays non-systematic variation of trace elements with indices of olivine fractionation is likely to be contaminated. Contrarily, the very systematic variations from Belingwe samples[5] may represent a pristine record (at least for some elements).

Huppert *et al.* also speculate about nickel sulphide deposits. Green and Naldrett suggested that some Canadian and Western Australian deposits might have assimilated sulphur from nearby sulphide-rich banded ironstones[6]. Huppert *et al.* consider that lava may have digested such sediments by thermal erosion. Sulphur isotope studies may help determine the value of their hypothesis — which could be useful in selecting exploration targets. Finally, Huppert *et al.* consider spinifex textures — the long plates of olivine that hang down in sheaves from the top surfaces of some komatiite flows. Experimental cooling rates necessary to reproduce these textures are much higher than rates allowed by conductive cooling models but convective cooling in turbulent flows would explain the textures nicely and Huppert *et al.* have produced good analogues of spinifex textures in Na_2CO_3 solutions.

What then for us stamp collectors? Clearly we must re-examine our data on the age and chemistry of komatiites and think again about the minor-element make-up of the mantle. Perhaps the recognition that contamination took place will help in resolving controversies about the extent of variation in chemistry of mantle source regions. More likely it will add to our uncertainty and send komatiitophiles scurrying back cheerfully to their field areas and to isotope laboratories. □

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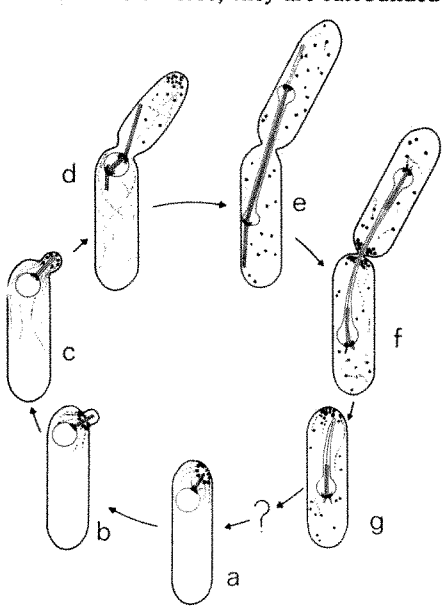
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Cell biology

A budding interest in the yeast cytoskeleton

from Jeremy Hyams

COMPARED with the avalanche of papers devoted to the genetics and molecular biology of the budding yeast, *Saccharomyces cerevisiae*, which has sometimes threatened to bury the pages of this and some other journals over the past few years, yeast cell biology has had a fairly thin time of it. The trouble is that yeast cells are just not designed for the job — they are small, their cytoplasm is dense and, to make matters worse, they are surrounded by a tough cell wall. Hence the curious situation that, whilst yeast actin has been purified and its gene cloned and sequenced[1], absolutely nothing has been known about its distribution or function during the budding cycle. Tubulin is somewhat better characterized, particularly in terms of the number and arrangement of spindle microtubules during cell division[2–4], but the role, and even the existence, of cytoplasmic microtubules has been far from certain. A recent pair of papers in *Journal of Cell Biology*[5,6] therefore marks a major step forwards in yeast cytology.



Schematic representation of the changes in actin and tubulin distribution in budding yeast during the cell cycle (a–g). Microtubules appear as straight lines growing from the spindle pole body which is embedded in the persistent nuclear envelope. Actin is shown either as black dots or as fine fibres. From ref.5.

What made this work possible was the isolation of monoclonal antibodies to yeast tubulin[7]. Anti-tubulin antibodies have been valuable tools for cell biologists for several years but conventional anti-tubulin antibodies raised against mammalian proteins do not cross-react with the microtubules of more primitive eukaryotes. On the other hand, the monoclonal antibodies to yeast tubulin appear to be universally cross-reactive. Using them and immunofluorescence microscopy, Kilmartin, Adams and Pringle have not only demonstrated the distribution of actin and tubulin during the yeast cell cycle in stunning detail but have also gone a long way to solving some of the old puzzles about the role of these proteins during budding[5,6].

Applied to the parent organism, the yeast anti-tubulin monoclonals generate intense staining both of the mitotic spindle and of extensive bundles of cytoplasmic microtubules (see the figure). In cells which have not yet formed a bud, cytoplasmic microtubules extend from the nucleus to the site from which the bud will emerge. Double staining with anti-actin and

fluorochrome-labelled phallotoxins, which bind specifically to F-actin, shows this region of the cytoplasm to be occupied by a ring of brightly staining actin dots. Simultaneous staining with Calcofluor reveals that the actin dots directly underly the ring of chitin which is laid down in the otherwise chitin-free cell wall and which defines the budding site.

As the bud emerges and enlarges the actin dots are displaced towards the bud tip at the same time as fine fibres of actin extend through both the bud and the mother cell. During this reorganization the spindle forms and elongates. Significantly, in view of the current speculation regarding the role of actin in chromosome movement, no actin is detected within the nucleus at any stage of the budding cycle of yeast. When the bud has reached its maximum dimensions the actin dots disperse through the cytoplasm but then concentrate in the neck region at the time of cytokinesis.

The changes in the distribution of actin are consistent with the suggestion that actin has a role in the localized desposition of cell-wall materials, first in the chitin ring and later at the tip of the bud. A similar pattern of staining in another fungus, *Uromyces phaseoli*, led to the suggestion that the dots of actin were microvesicles coated with filaments having the same dimensions as F-actin ('filasomes')[8]. In *S. cerevisiae*, cytoplasmic microtubles may direct similar vesicles, containing cell-wall precursors, to their sites of deposition[2] or may simply serve to orient correctly the spindle for its later extension into the bud.

The introduction of effective immunofluorescence procedures to *S. cerevisiae* adds the yeasts to the list of organisms in which fundamental cell-biological problems can now be studied. Remembering the sophisticated genetics that has already been developed for yeasts, the potential of these simple organisms seems to be limitless. □

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Cosmology

Fluctuations in microwave background radiation

from M.S. Longair

NEARLY twenty years after the discovery of the microwave background radiation by Penzias and Wilson, all but the most sceptical cosmologists are convinced that the hot big-bang model provides the most promising framework within which to study the more profound problems of cosmology. It has long been recognized that directional fluctuations (ΔI) in the intensity (I) of the microwave background radiation should be a powerful way of investigating a wide range of properties of the hot big-bang model. The detection of such fluctuations has, however, proved to be extremely difficult and it is only with the paper of Birkinshaw, Gull and Hardeback on page 34 of this issue that it is possible to say with confidence that at least one type of small-scale fluctuation has been observed.

The only anisotropic signal unambiguously detected so far at a level of about $\Delta I/I \sim 10^{-3}$ is associated with the motion of the Earth relative to the frame of reference in which the background is isotropic. Otherwise there are only upper limits to the intensity fluctuations, the most sensitive at a level of about $\Delta I/I \sim 10^{-4}$. The problem is that, at this level of sensitivity, very small instrumental effects can introduce spurious fluctuations while, in addition, the atmosphere introduces fluctuations due to astronomical 'seeing' in the radio waveband.

Birkinshaw *et al.* have searched for the small dips or decrements in the microwave background radiation first predicted by Sunyaev and Zel'dovich in 1968 on the grounds that the spectrum of the microwave background could be distorted by quantities of hot gas along the line of sight. If the gas were very much hotter than the black-body radiation, the hot electrons would heat the radiation by Compton scattering and, in a simple approximation, the whole microwave background spectrum would be shifted to a slightly higher frequency while the total number of photons will be unchanged. Thus, if the microwave background radiation traverses a region of hot gas on its way to the observer, there will be a decrease in the intensity in the long-wavelength (Rayleigh–Jeans) region of the Planck spectrum and an increase in the high-frequency (Wien) region.

Since X-ray observations with the Uhuru, Ariel V and Einstein satellite observatories have shown that there are vast quantities of hot gas at temperatures of 10^7–10^8 K in the direction of rich clusters of galaxies, Birkinshaw *et al.* turned the 40-m telescope of the Owens Valley Radio Observatory, California, in those directions in their search for intensity fluctuations. As a result, after years of marginal detections and several false starts, fluctuations at a high level of significance have been observed in the direction of three rich clusters of galaxies. The relative intensities of these fluctuations range from about 5×10^{-4} in the case of cluster 0016+16 to about 2×10^{-4} in the case of the Abell clusters 665 and 2218. It is particularly impressive that these values are all claimed at or above the 7σ confidence level — a striking measure of the sensitivity of the observations.

Astrophysically, this result is important because the fluctuations provide information about the hot gas which has modulated the microwave background in some particular direction in the sky. Indeed, the magnitude of the decrement is determined solely by the optical depth for Compton scattering by the hot gas, which is proportional to the electron density N_e, the electron temperature T_e and the dimension of the cloud in the line-of-sight. Complementary information about the hot gas can be found from X-ray observations of the clusters. The temperature can be obtained from the shape of the spectrum, which for energetic X rays is roughly proportional to $\exp(-h\nu/kT_e)$ while the total intensity of X-ray emission is proportional to N_e^2, to $T_e^{-1/2}$ and to the volume of the cloud.

Thus, if adequate spatial resolution is available at radio and X-ray wavelengths and the distribution of gas in the cluster is reasonably well behaved, the physical conditions are overdetermined so that the physical size of the gas cloud in a cluster can also be found. That, in turn, opens up the exciting prospect of a new route to the determination of Hubble's constant since we now know the physical size of a 'measuring rod' at a known redshift.

But this achievement is only the beginning of what Birkinshaw and his colleagues foresee as a new epoch in observational cosmology. The Sunyaev–Zel'dovich effect was probably the easiest of challenges; if sensitivity can be improved by an order of magnitude, a whole new range of phenomena will come within our observational grasp.

Among the most important goals is the discovery of fluctuations predicted to be associated with the initial collapse of protogalaxies and protoclusters. According to all acceptable theories of the origin of structure in the Universe, the growth of the fluctuations of ordinary matter from which galaxies and clusters have formed became possible only after matter and radiation were decoupled at a redshift of about 1,500, corresponding to an epoch of 10^6–10^7 years after the big bang. The requirement that galaxies and clusters should now exist results in lower limits to the material density fluctuations at that early epoch and thus, because of Compton scattering, to radiation anisotropies that should now be apparent as fluctuations in the microwave background in the range

$\Delta I/I \sim 10^{-4}$–10^{-5}. Observations of such fluctuations would provide strong support for the hypothesis that structures formed by collapse which began when matter and radiation decoupled. This is a rather general argument which remains valid even in recent scenarios of galaxy formation involving massive neutrinos and other weakly interacting particles that may contain most of the mass of the Universe.

Other sources are expected to produce fluctuations at about the same level. Thus the reheating of gas at a late epoch should induce fluctuations, while the passage of radiation through large collapsing structures can cause fluctuations by the gravitational redshifting produced in the process. Even the hot gas in rich clusters, now detected by Birkinshaw *et al.*, must yield fluctuations on a fine scale, and these may well limit the detectability of genuine primordial fluctuations.

Such an approach is, nevertheless, one of the few that can provide observational information about the epochs between about 10^7 and 2×10^9 years. Already, the Cambridge radioastronomers have developed proposals for achieving the extra factor of 10 in sensitivity needed to tackle these more ambitious programmes. It is hard to disagree with their view that the next decade will be a period when these exciting and fundamental observations become an integral part of astrophysical cosmology. □

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Astronomy

Black holes, accretion discs and Seyfert galaxies

from C. Martin Gaskell

A LARGE consortium of European astronomers has recently published the third instalment of results of their ongoing programme monitoring the nearby Seyfert 1 galaxy NGC4151 with the International Ultraviolet Explorer satellite (IUE)[1]. Results have already been published from similar programmes monitoring other Seyferts (the lowest-luminosity quasars) but NGC4151, the first Seyfert to be discovered[2], has always received more ground- and space-based attention than any other. Considerable publicity before publication by the Royal Greenwich Observatory alleged that the 'mass of the black hole' in NGC4151 had been measured. Although this claim captured the imagination of the British press, the truth is that neither the IUE consortium nor anybody else has proved that there is a black hole in NGC4151 or any other quasar-like object. The mass estimated for the central object is in fact not new. An almost identical value was derived 25 years ago[3] and the basic method employed has already been used innumerable times over the last couple of decades. It is widely recognized that the method could be giving a wrong answer however. The IUE consortium claims to have made 'remarkable progress' in understanding the nuclear energy source of NGC4151, but, interesting as their observations are, unfortunately they neither prove nor disprove the current standard theory that quasars and similar objects are empowered by accretion of matter in a disc onto a massive black hole. Much of the interpretation of changes in the ultraviolet spectrum is probably wrong.

How do you estimate the mass of a quasar nucleus? The most direct way is to use Newton's law of gravity. Since one obviously cannot follow a single test particle around an orbit one has to use the integrated properties of the ensemble of cloudlets in the broad-line region and make certain assumptions. The most usual assumption is that the cloudlets are in bound orbits with an isotropic velocity distribution. To deduce the central mass one needs to know the average velocity and the average distance. The velocities of broad-line clouds have been well known since the pioneering work of Seyfert[4] in 1943. The Doppler widths of the broad lines imply velocities of several per cent of the speed of light. The sizes of broad-line regions were first calculated from photoionization considerations[3,5] but the subsequent discovery, in 1967, of variability of emission lines in Seyferts[6,7] permitted an independent estimate of the size of the broad-line regions from light-travel-time considerations[8]. The two methods are in satisfactory agreement. It is the latter method that Ulrich *et al.*[1] have used. Woltjer[3] estimated the central mass of NGC4151 to be 3×10^7 solar masses as long ago as 1959 and this sort of mass has been almost universally accepted by workers in the field ever since. Clearly the IUE observers have not made a new contribution here.

Are we confident that we know the mass of the central object fairly well? The answer is no. The problem with the standard newtonian calculation described above is that the assumption of bound orbits (or at least of cloud motions dominated by gravity) is not valid. There is abundant evidence for the outflow of gas from quasars and Seyferts in general (this has already been reviewed in a recent *News and Views*[9]) and in NGC4151 in particular[10]. If the cloud motions are dominated by radiation pressure (causing the outflow), rather than by gravity, the assumption of orbital motion is clearly invalid and the mass can easily have been overestimated or underestimated by an order of magnitude.

Why do we think that quasars contain massive black holes? All the arguments for the existence of massive black holes in active galactic nuclei are theoretical. If the mass of the central object is indeed 3×10^7 solar masses this does not automatically prove that it is a black hole rather than, say, a supermassive star or a spinar. Nonetheless, accretion of matter onto a massive black hole has long been considered the most likely source of energy in quasars[11]. If the accreting matter has any angular momentum at all it will collapse into a flattened configuration (an 'accretion disc'). Such discs are known to exist around many collapsed stellar objects (the dwarf novae, cataclysmic variables and so on) in our own Galaxy. The standard model of quasars is currently an accretion disc surrounding a massive black hole.

Although such discs are receiving considerable theoretical attention, the only observational hint that they exist around quasars is a slight curvature in the spectral energy distribution in the optical–ultraviolet region. Shields[12] has proposed that this could be due to thermal emission from an accretion disc superimposed on an underlying power-law non-thermal continuum. But this is by no means the only interpretation of the continuum energy distribution in quasar spectra. The interpretation is complicated by the presence of many weak blended Fe II lines[13] and uncertainties in the amount of reddening by dust in the quasars. The excess emission around 3,000 Å (the so-called '3,000 Å bump') is almost certainly Balmer continuum and blended Fe II line emission[14,15]. The remaining curvature in the ultraviolet–optical spectra of quasars can be understood in terms of standard synchrotron-self-Compton theory without invoking any additional thermal component at all[16]. If there is a thermal excess it does not have to be from an accretion disc — it could be from the

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photosphere of a massive superstar[17] or from very-high-density broad-line-region gas close to the central object[18].

Observations of time variability of the continuum spectral shape of Seyferts and quasars are going to be important in understanding the origin of the continuum and the observations by Ulrich *et al.*[1] and others[19,20] have shown interesting changes in the ultraviolet continuum of NGC4151 and other Seyferts. IUE observations alone, however, just do not provide enough wavelength coverage and the changes reported are not, I believe, true changes in the continuum shape but rather changes in the relative intensity of the Fe II emission at 3,000 Å (that is, the 3,000 Å bump). This is probably the cause of the otherwise puzzling result of Ulrich *et al.*[1] that the C IV 1,549 Å emission-line variability is better correlated with the continuum emission at 2,500 Å than with the continuum further into the ultraviolet (that is, nearer to the relevant ionization edges), and other inconsistencies which they note. To measure the true continuum one needs to cover many octaves in frequency and it is to be strongly hoped that future IUE observations of NGC4151 (and other Seyferts) will be scheduled with simultaneous longer-wavelength optical and infrared observations (and for that matter X-ray observations as well).

Although our picture of NGC4151 and other active galactic nuclei is still very fuzzy and little has actually been proved, it must be said that everything is still consistent with such objects having at least one massive black hole in their centres (and some may have two[21]). Similarly there is no evidence so far that quasars do not have accretion discs powering them, but it would be nice to have proof that they do. □

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Protein structure

Oxygen carriers of a third kind

from K.E. van Holde

HAEMOGLOBIN is not the only way to transport oxygen in a circulatory system. Some invertebrate phyla use altogether different proteins to serve this function. These include the haemerythrins, utilized by sipunculids, brachiopods and a few other invertebrates, and the haemocyanins, found in a wide variety of molluscs and arthropods. The structures of many haemoglobins, and even one haemerythrin[1], have been determined to high resolution by diffraction techniques, but until the paper of Gaykema *et al.*[2] on page 23, such information has been wholly lacking for the haemocyanins. This hiatus has seemed almost paradoxical, for the haemocyanins were the first proteins to be studied in detail by biophysical techniques, notably in some of the earliest ultracentrifuge studies of Svedberg and his collaborators[3].

The problem of how to bind oxygen in a way that is reversible receives a radically different solution in haemocyanins than it does in haemoglobins and haemerythrins. Rather than using iron as an oxygen ligand, haemocyanins employ pairs of antiferromagnetically coupled copper ions[4,5]. Such copper pairs are found in a number of other proteins, but for none of these is the detailed structure known. Thus the determination, to a resolution of 3.2 Å, of the structure of the haemocyanin of the spiny lobster, *Panulirus interruptus*, is of major importance.

The native molecule, which is one of the simpler haemocyanins, exists as a hexamer of two kinds of very similar subunits, each of molecular weight (MW) about 75,000. The entire hexameric structure has now been determined by Gaykema *et al.*[2], making it one of the largest proteins to be analysed by X-ray diffraction. The subunits are found to be arrayed with 32 symmetry; two trimers, related by 2-fold axes, are stacked face to face. Each subunit consists of three domains of approximately equal size. The second of these domains carries the copper pair. Each copper ion is bound by three residues, two of which are definitely histidines. The third ligand is probably also a histidine, but confirmation will require further sequencing.

The structure determined by Gaykema *et al.* seems to be that of deoxyhaemocyanin but studies of a subunit of *Limulus* oxyhaemocyanin are under way in the laboratory of W. Love, at Johns Hopkins University[6]. It will be of considerable interest to see how the oxy- and deoxy-structures compare, for haemocyanins are allosteric proteins, exhibiting both cooperativity in oxygen binding and heterotropic allostery, including a pronounced Bohr effect. So far, our only model for these functions has been haemoglobin (haemerythrin appears to be non-cooperative[7]); it is conceivable that similar effects will be found to be produced by quite dissimilar mechanisms in a protein as different as haemocyanin. This would represent an interesting example of convergent evolution of protein function.

The structure found for the haemocyanin seems wholly unrelated to that of haemerythrin or any haemoglobin, so haemocynanin must have a different ancestry. Is it shared with tyrosinases, ceruloplasmin and laccase, all proteins with a similar binuclear copper centre? Although the three-dimensional structures of these proteins are unknown, their amino acid sequences have certain motifs in common, suggesting similar modes of copper binding. In all three proteins there exist pairs of histidines separated by three amino acids, resembling those now identified among the copper ligands of haemocyanin. Confirmation of the hypothetical ancestry may be difficult, however, for the haemocyanins have presumably been evolving independently for something on the order of 500 million years. Indeed, a comparison of the complete sequence of a spider haemocyanin with *Neurospora* tyrosinase gave no evidence of homology[8].

Gaykema *et al.* point out a peculiar aspect of the *Panulirus* haemocyanin structure. Its carboxy-terminal domain contains a seven-stranded antiparallel β-barrel, remarkably similar in topology to the β-barrels in (Cu–Zn) superoxide dismutase and immunoglobulins[9]. Since the amino acid sequences are entirely different, they argue that this may simply be a very stable structure which has evolved independently in different proteins.

A final puzzle, which may be clarified by further structural studies, is the relationship between arthropod and molluscan haemocyanins. The oxygen-binding site of both types seems quite similar but their gross molecular architecture is entirely different. All known arthropod haemocyanins are built of 75,000-MW chains, arranged in hexamers, as in *Panulirus*. These hexamers are often combined to form even larger structures. On the other hand, the polypeptide chains of molluscan haemocyanins are much larger; units of MW about 400,000, containing eight globular domains and eight oxygen-binding sites, are commonly observed[4,5]. Gaykema *et al.* suggest that a molluscan domain of ~ 50,000 MW may correspond to two of the three 25,000-MW domains observed in the *Panulirus* structure, one carrying a binuclear copper site. Thus, it is conceivable that molluscan and arthropod haemocyanins arose via separate evolutionary events from some common copper-protein ancestor.

The new structural studies, combined with a number of sequence determinations now underway, promise to broaden our perspectives concerning the mechanisms that organisms have evolved to transport oxygen. □

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Emplacement and cooling of komatiite lavas

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We propose that komatiite lavas were emplaced as turbulent flows, accompanied by vigorous forced convection with cooling rates often in excess of hundreds of °C h^{-1}. They melted and assimilated up to 10% of the ground over which they flowed, forming deep channels. Nickel sulphide mineralization may have resulted from incorporation of sulphur-rich sediment. After emplacement, high cooling rates persisted, resulting in spinifex textures due to compositional convection.

KOMATIITES are believed to represent ultramafic liquids which erupted at temperatures of 1,400–1,700 °C with estimated viscosities in the range 0.1–1 Pa s, and with MgO contents of 18–35%[1–3]. Such lavas are mostly of Archaean age. They indicate that thermal conditions of the early Earth were significantly different from those of today and provide insights into the composition of the Archaean mantle. They commonly exhibit spectacular spinifex textures and internal layering[4], and some are also the host to important nickel sulphide mineralization[5]. Interpretation of their chemical compositions, petrological and geological features and mineralization requires an understanding of their eruption, emplacement and cooling. As a contribution to this understanding, we present both a fluid dynamical analysis of the flow and cooling of komatiites and experimental studies which simulate the formation of spinifex texture in ponded komatiite lavas.

Recent basalts have eruption temperatures usually below 1,250 °C and viscosities >50 Pa s; almost all of them flow by laminar shear at low Reynolds number and cool by conduction. We propose that, because of their much lower viscosities, komatiites would have flowed turbulently at high Reynolds number, and would have cooled initially by forced convection. We suggest that, after emplacement, komatiite lavas still cooled rapidly by thermal convection and demonstrate, by experimental studies on cooling of saturated aqueous solutions, that convective effects can strongly influence the development of the characteristic spinifex textures.

Magma ascent

Magma ascends to the surface because of its buoyancy[6,7]. At high Reynolds number, Re, the two-dimensional discharge rate, Q, from a long fissure of width d can be estimated as

$$Q = \left(\frac{g\Delta\rho}{k\rho_m}\right)^{1/2} d^{3/2} \qquad (1)$$

where g is gravity, $\Delta\rho$ is the assumed constant density difference between the magma and the lithosphere overlying its source, ρ_m is the magma density, k is a friction coefficient which can assume values between ~0.01 and 0.06 depending on the surface roughness of the fissure[7], and the Reynolds number is

$$Re = Q/\nu \qquad (2)$$

where ν is the kinematic viscosity. In principle, in order to account for the density variation in the lithosphere, the compressibility of the melt and the tortuous nature of the fissure, a more elaborate treatment than that leading to equation (1) is required. However, the accuracy of equation (1) is sufficient for our calculations. At shallow depths in the lithosphere, the value of $\Delta\rho$ is ~300 kg m^{-3}, whereas at depths of 100 km the value of $\Delta\rho$ has been estimated as 100 kg m^{-3} (ref. 8).

Table 1 gives the values of Q, flow velocity $v = Q/d$, and Re for a plausible range of natural fissure widths. The komatiite is assumed to have a viscosity of 0.3 Pa s, a density of 2,800 kg m^{-3} and we have set $\Delta\rho$ to a conservative value of 100 kg m^{-3}. The value of 0.03 has been used for the friction coefficient. Table 1 shows that even for the narrowest fissure (0.3 m), Re greatly exceeds the critical value (equal to ~2,000) required for turbulent flow. Komatiites are thus likely to have flowed turbulently up the fissure before eruption. This should be compared with the largest historic eruptions of basalts, for which Q and Re are less than 10 m^2 s^{-1} and 600 respectively[7].

Table 1 The two-dimensional flow rate, Q, the vertical velocity, v, and the Reynolds number, Re in a fissure of width d

d(m)	Q(m^2 s^{-1})	v(m s^{-1})	Re
0.3	0.56	1.9	5.2×10^3
1.0	3.4	3.4	3.2×10^4
3.0	17.8	5.9	1.6×10^5
10.0	108	10.8	1.0×10^6

Lava emplacement

A fundamental feature of a turbulent flow is that the heat transfer is much greater than in a laminar flow, where heat is lost entirely by conduction. In turbulent flows, mixing maintains a uniform temperature throughout the span of the flow and greatly increases heat transfer to the surroundings. Thus, in turbulent komatiite flows the ground surface would be quickly heated up to temperatures of 1,600–1,700 °C. In greenstone belts, komatiites often flow over rocks, such as tholeiitic basalt and sediment[9,10], which have substantially lower melting temperatures. We suggest that komatiites could have melted and assimilated this underlying rock.

Our model, a geometric sketch of which is presented in Fig. 1, is related to that proposed by Hulme[11,12], who argued that the sinuous rilles on the Moon were caused by thermal erosion of lava channels by turbulent lunar flows. We consider flow on a shallow slope beneath a deep body of water. Both floor rocks and water have initial temperature T_0, which is taken to be 0 °C. The heat transfer from the komatiite, due to forced, turbulent convection, melts some of the ground which is then

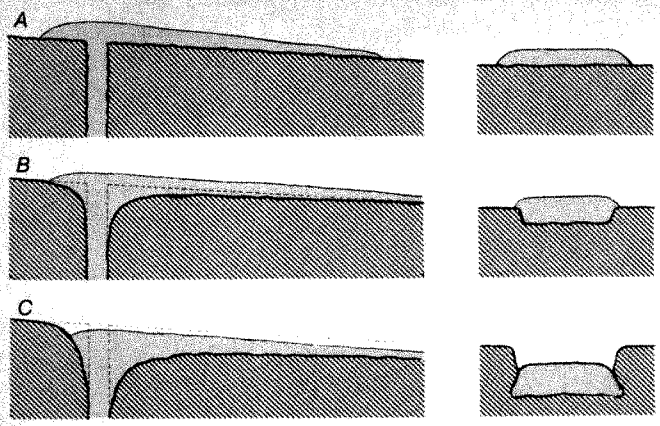


Fig. 1 Longitudinal and cross-sectional sketches of the fissure and lava flow showing the melting of the ground and the formation of an erosional channel with time.

assimilated. Efficient cooling by the water above the flow forms a thin crust on top of the lava and prevents any mixing between the two. As the lava cools, crystals form within it, which decreases the rate of cooling. The komatiite is assumed to flow in the form of a gravity current of high Reynolds number over a slope which is sufficiently small that its effects can be neglected. The thickness of the current, h, can then be determined[13] by

$$h = (\tfrac{1}{2}Q^2\rho_l/g\delta\rho)^{1/3} \quad (3)$$

where $\delta\rho$ represents the density difference (1,800 kg m^{-3}) between the komatiite and seawater. Table 2 presents the flow thickness, velocity, u, and Reynolds number for different values of Q. A gravity current with a Reynolds number greater than ~500 is fully turbulent[14]. The values in Table 2 indicate that this is the case for all komatiite flows.

At steady state the melting rate is given by[11,15]

$$\frac{dS}{dt} = \frac{H}{\rho_g[c_g(T_m - T_0) + L_g]} \quad (4)$$

where H is the heat transfer rate to the ground, ρ_g is the density of the ground, c_g is the specific heat of the ground, T_m is both the melting temperature of the ground and the solidification temperature of the komatiite and L_g is the heat of fusion of the ground. The heat transfer is related to T_l, the lava temperature, and T_m through a heat transfer coefficient, h_T, by[15]

$$H = h_T(T_l - T_m) \quad (5)$$

An approximation to the heat transfer coefficient can be obtained by modifying empirical relationships for turbulent pipe flows[15]. This leads to

$$h_T = 0.018\frac{k_l}{h}Pr^{0.4}Re^{0.8} \quad (6)$$

where k_l is the lava conductivity and Pr its Prandtl number.

Table 2 shows that transfer coefficients of between 500 and 1,000 W m^{-2} °C^{-1} result for flow rates in the range 0.5–100 m^2 s^{-1}. This variation in the heat transfer coefficient is fairly small and for simplicity we have taken $h_T = 750$ W m^{-2} °C^{-1} in the subsequent calculations. With the values of physical parameters listed below inserted into equations (4) to (6), the melting rate of the ground near the source is calculated to be 8.1×10^{-5} m s^{-1} (7 m per day), confirming that thermal erosion was a major effect during the emplacement of komatiites. (The physical parameters used were: $k_l = k_c = 1$ W m^{-1} °C^{-1}; $c_l = c_g = 730$ J kg^{-1} °C^{-1}; $\rho_l = 2{,}800$ kg m^{-3}; $\rho_g = 2{,}700$ kg m^{-3}; $L_g = L_x = 5 \times 10^5$ J kg^{-1}; $T_0 = 0$ °C; $T_m = 1{,}200$ °C; T_l $(x = 0) = 1{,}600$ °C; $\theta_0 = T_l$ $(x = 0) - T_m = 400$ °C; and $Pr = 219$.)

Table 2 Lava flow thickness, h, its velocity, u, the Reynolds number, Re, the heat transfer coefficient, h_T, and the coefficient modified by crystallization, h_T^x, as a function of Q

Q(m^2 s^{-1})	h(m)	u(m s^{-1})	Re	h_T (W m^{-2} °C^{-1})	h_T^x (W m^{-2} °C^{-1})
0.5	0.27	1.8	4.7×10^3	498	238
1	0.43	2.3	9.3×10^3	543	259
10	1.99	5.0	9.3×10^4	739	353
100	9.26	10.8	9.3×10^5	1,000	477

Table 3 The cooling rate at the source, $\dot{T}(0)$, the erosion rate at the source, $\dot{S}(0)$, and the length scale over which the temperature of the lava decreases, D^{-1}, as a function of Q

Q(m^2 s^{-1})	$\dot{T}(0)$ (°C h^{-1})	$\dot{S}(0)$ (m per day)	D^{-1} (km)
1	1,300 (2,730)	3.33 (6.98)	2.9 (1.4)
10	280 (590)	3.33 (6.98)	28.5 (13.6)
100	61 (127)	3.33 (6.98)	285 (136)

The second entry, in parentheses, is the value if latent heat effects are set equal to zero.

A thin crust forms at the contact with seawater. At the top of the crust the temperature is T_0, while at the bottom it is T_m. The crustal thickness, a, is determined from the fact that in a steady state the conductive heat transfer through the crust equals the heat transfer from the lava given by equation (5). Thus

$$a = k_c(T_m - T_0)/h_T(T_l - T_m) \quad (7)$$

where k_c is the conductivity of the crust. Near the source $a \approx 4$ mm and increases away from the source as the lava temperature decreases.

Cooling of the lava

Heat loss from the lava causes crystallization, which releases latent heat, L_x. A quantitative heat budget calculation indicates that the lava temperature as a function of downstream distance x is given by

$$T_l = T_m + \frac{D\theta_0}{(E\theta_0 + D)\,e^{Dx} - E\theta_0} \quad (8)$$

where θ_0 is the difference between the temperature of the lava at source and the melting temperature of the ground, $D = 2h_T^x/(\rho_l c_l Q)$,

$$E = \frac{h_T^x}{Q\rho_g[c_g(T_m - T_0) + L_g]} \quad (9)$$

and

$$h_T^x = \frac{h_T}{1 - L_x x'(T)/c_l} \quad (10)$$

with $x'(T) = -\frac{1}{625}$ °C^{-1} representing the rate of change of crystal fraction with temperature[16]. For $Q = 10$ m^2 s^{-1}, the lava temperature as a function of distance is plotted in Fig. 2A. The temperature decreases with a length scale given by D^{-1}, which is evaluated for other flow rates in Table 3. If the lava increases the ground temperature to T_m but does not melt it, its temperature is given by $T_l = T_m + \theta_0 e^{-Dx}$, also plotted in Fig. 2A.

The cooling rates of the lava flow for $Q = 10$ m^2 s^{-1} are plotted in Fig. 2B; one curve incorporates latent heat release due to crystallization while the other does not. With latent heat effects the cooling rate is 280 °C h^{-1} near the source and decreases steadily away from it. Initial cooling rates for other flow rates are given in Table 3.

These calculations assume that crystallization occurs in thermodynamic equilibrium, although experimental studies[17,18] on magnesian-rich basic melts show that rapid cooling rates can

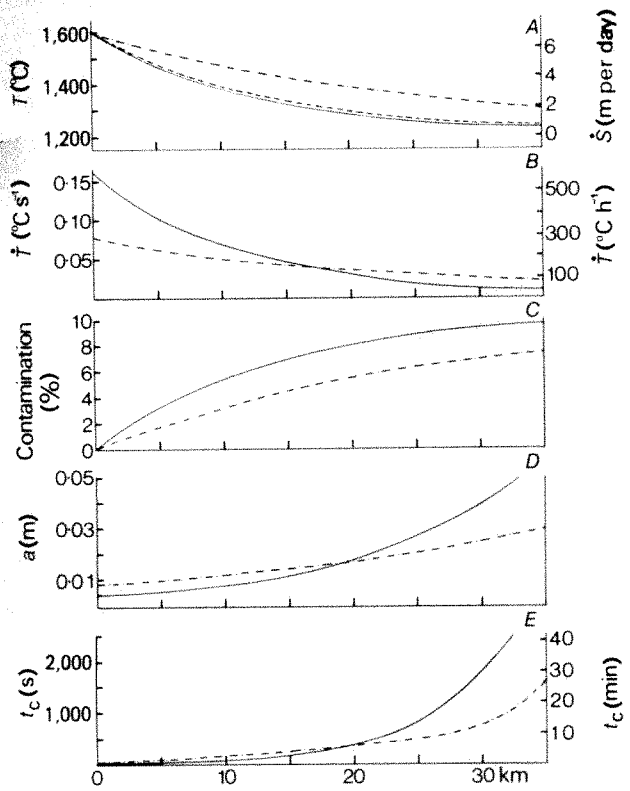


Fig. 2 *A*, The temperature of the lava, T, and the erosion rate of the ground, S. ——, With melting; – – –, without melting. *B*, The cooling rate; *C*, the percentage of contamination; *D*, the crustal thickness, a; and *E*, the time scale for the crust to form; all as a function of distance from the source. In *A*–*E* the dot–dash (– · –) line incorporates the latent heat release due to crystal formation, while the solid line omits this effect ($L \equiv 0$).

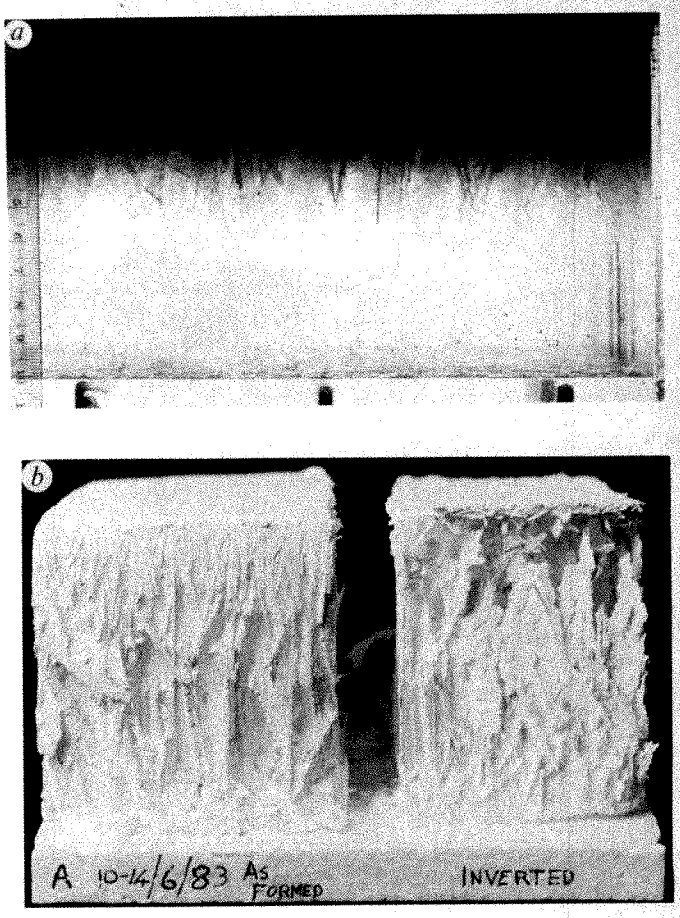


Fig. 3 Photographs of an experiment in which aqueous Na_2CO_3 was cooled from above. *a*, Photograph with back-lighting after 1 h 38 min. Note the growth of long crystals with nearly vertical orientation, followed by a layer of eutectic with a sharp horizontal boundary. The scale on the left indicates the distance (in cm) from the cooled roof. *b*, The subsequent crystal block after ice was allowed to melt and run out of the upright (left) and inverted (right) halves of the block.

cause large undercoolings. Extrapolation of Donaldson's experimental data[18] to cooling rates of several hundreds of °C per hour suggests undercoolings of 50–70 °C. The cooling rates in excess of 10^3 °C h^{-1}, for small Q, might result in considerable delays in olivine nucleation, with the extreme case of zero crystallization. Table 3 shows the results of calculations neglecting crystallization completely; cooling rates and melting rates increase as the undercooling increases.

As the melting rate of the ground is proportional to $T_l - T_m$, it has the same functional form as T_l (Fig. 2*A*). Our calculations indicate that one hour after eruption, with the flow rate $Q = 10\ m^2\ s^{-1}$, the channel depth at source would be 14 cm, while 15 km from the source it would be 8 cm. After a week the depth at source would be 23 m and 15 km from source it would be 13 m. If no crystallization occurred, the depths would be just over twice these values. If the lava flow were 250 m wide, the volume erupted after one hour and one week would be 9×10^6 and $1.5 \times 10^9\ m^3$, respectively. These volumes are comparable to small- and medium-sized basalt eruptions.

Assimilation of the melted ground contaminates the lava, as indicated in Fig. 2*C*. The contamination rises steadily to an asymptotic value of 10.4%, independent of the flow rate, and could significantly change the trace element and isotopic composition of the lava, although it would have only minor effects on the major element composition.

The crustal thickness, given by equation (7) and plotted in Fig. 2*D*, is of the order of a few centimetres, consistent with the geological evidence that komatiites often show an upper quench glassy zone of this magnitude[4]. The time scale for this crust to grow (Fig. 2*E*) is quite short and indicates that if at any time the crust is broken up by the turbulent flow beneath it and falls into the flow, a new crust forms fairly rapidly. Thus a steady-state crust caps the flow during almost all the emplacement period.

Post-emplacement processes

Once the supply of magma has ceased, the komatiite can be expected to drain into any available depressions, which would include the channel incised by thermal erosion. In many lavas only part of the cooling will have been accomplished during active flow and the ponded lava will continue to cool and crystallize. If the crust is thin, theoretical calculations (which will be presented in detail elsewhere) show that the ponded komatiite will continue to cool by thermal convection. By balancing the conductive heat flux across the crust with the convective flux in the interior, we obtain initial cooling rates of tens to hundreds of °C per hour. As the crust thickens from being a few centimetres thick, the lava temperature decreases and eventually turbulent convection will decrease in vigour. At this stage the heat budget is dominated by a balance between the conductive flux across the crust and latent heat release. We envisage that granular olivines suspended in the lava will now fall out to form the peridotitic cumulate layer, typical of the lower parts of many komatiites[10].

In order to simulate the development of spinifex texture, we crystallized saturated aqueous solutions by cooling from above. Figure 3 shows the results of cooling an aqueous solution of

12.3% anhydrous Na_2CO_3 in a cubical container initially at 31 °C by circulating alcohol at −30 °C through the top plate. Initially, vigorous thermal convection occurred and small dendritic crystals of hydrated Na_2CO_3 nucleated on the roof. At first the crystals showed a wide range of orientations with respect to the roof (Fig. 3*a*). As the experiment progressed, however, the crystals increasingly became oriented perpendicular to the roof (Fig. 3*b*). At the same time, a stagnant pool of less dense, residual fluid formed between the growing crystals due to compositional convection[19]. The tips of the crystals extended beyond this layer into the saturated, convecting fluid beneath. Experiments on other saturated solutions of Na_2SO_4 and KNO_3 mirrored these features, confirming that the general behaviour is not dependent on composition.

Geological interpretations

Our analysis shows that komatiites can become significantly contaminated by assimilation of the underlying rock (Fig. 2*C*). We emphasize three points concerning the contamination process. First, floor rocks will be quickly melted and efficiently mixed into the turbulent flow. Xenoliths would not be expected to survive. Second, the chemical signature of contamination would not be extreme; only elements strongly concentrated in the contaminant and depleted in the komatiite would be noticeably affected, and the evidence for this would be most apparent in measurements of the ratios of elements with different compatibilities. Third, the amount of contamination is strongly dependent on flow conditions and distance downstream. As most eruptions probably fluctuate in intensity, the amount of contamination is likely to be very variable even within one flow. Non-systematic variations with indices of olivine fractionation would be anticipated for those elements that are sensitive indicators of contamination.

An application of the thermal erosion model relates to the origin of nickel sulphide mineralization, which we suggest could have been formed by komatiites melting underlying sulphur-rich sediments. Contamination of 10% of a sediment containing 6% sulphur could increase the sulphur content of the lava by 0.6%. The sulphur forms an immiscible sulphide liquid which extracts nickel from the komatiite and segregates to the base of the flow[5]. The sulphide liquid could separate effectively even from the turbulently flowing lava because of its high density (~4,500 kg m^{-3}), especially if it coagulated to form large drops with high settling velocities.

At Kambalda in Western Australia, the nickel sulphide ores are found at the base of komatiite flows where the lavas are thickest[9]. The long linear depressions which the ores occupy have been interpreted as pre-existing features down which the lavas were channelled. However, it would then be expected that sediment would accumulate in the channels and be thickest in these areas, whereas sediment rarely underlies the thickest komatiite flows and nickel sulphide is present there instead[20].

This antithetic relationship between the occurrence of sediment and ore is consistent with the suggestion that the depressions are thermal erosion channels where the lava has digested the sediment and cut into underlying basalt. The model also avoids the problem of trying to explain why highly magnesian melts carry such high contents of sulphur[20]. Thus, this model explains many of the features of mineralization, such as the regional association of sediment and ore-bearing komatiites, evident throughout the Western Australia nickel province. It remains to be seen, however, whether the model is of more general applicability.

Our work also relates to the origin of the characteristic textures and layering of komatiite flows[21]. Skeletal olivine morphologies and mineralogical peculiarities, such as crystallization of clinopyroxene, appear to demand either high rates of cooling or melts undercooled by hundreds of °C[17,18,22]. Cooling rates, based on conductive models[23], are orders of magnitude less than the rates required by experimental studies of olivine growth[17,18]. Thus, it is difficult to see how the slow cooling rates characteristic of conductive models can cause these textures. Our analysis of the post-emplacement phase, combined with Donaldson's experimental results[17], indicates that rapid cooling rates and large undercoolings can be achieved. Our experiments also indicate that compositional convection can significantly influence the morphology of crystals growing from the roof. Skeletal and dendritic crystal morphologies and metastable crystallization could be a consequence of both thermal and compositional convection.

Our study suggests that during flow, high cooling rates cause either pre-existing phenocrysts to grow, or high nucleation rates. Granular equant olivines remain suspended during turbulent flow and initially on emplacement, when thermal convection might occur. As convective stirring diminishes in vigour, the olivines settle out to form a cumulate layer. Olivine crystals nucleate on the roof and at first grow as dendrites in many orientations. However, compositional convection causes a pool of differentiated melt to form between the crystals beneath the roof. As the pool develops, the tips of olivine crystals grow preferentially at the margins, as in the experiment, and the olivines increasingly grow perpendicular to the roof.

We conclude that komatiites probably flowed turbulently and cooled in part by convection. One consequence of this behaviour is that komatiites can melt and assimilate underlying rock to form deep thermal erosion channels. Assimilation of sulphur-rich sediments may result in the formation of nickel ore deposits. After emplacement, komatiites can continue to cool by thermal convection. Nucleation of crystals on the roof results in the development of spinifex texture. All these processes have important influences on the development of the characteristic geology, textures, geochemistry and mineralization of these rocks.

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3.2 Å structure of the copper-containing, oxygen-carrying protein *Panulirus interruptus* haemocyanin

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For the first time, the three-dimensional structure of a member of the copper-containing class of oxygen-carrying proteins—the haemocyanins—has been determined. The structure of Panulirus interruptus *haemocyanin at 3.2 Å resolution shows that each subunit of the 450,000-molecular weight hexamer is folded into three domains, and that the binuclear copper site is situated in the centre of the second domain. Amino acid sequence comparisons suggest that the polypeptide fold of* P. interruptus *haemocyanin is common to all arthropod haemocyanins.*

OXYGEN is transported in living organisms by three completely different classes of proteins: haemoglobins, haemerythrins and haemocyanins. Several three-dimensional structures, and numerous amino acid sequences are known for haemoglobins and haemerythrins[1–7]. Haemocyanins have, so far, been much less well characterized at the atomic level. However, this picture is altering rapidly as recently a wealth of haemocyanin sequence information has become available[8–19]. Here the folding of the polypeptide chain of a haemocyanin molecule is presented for the first time.

Haemocyanins are large, non-haem, copper-containing proteins which occur freely dissolved in the haemolymph of many invertebrate species. Two major classes exist: molluscan and arthropodan haemocyanins, which have completely different molecular architectures. Arthropodan haemocyanins consist of hexamers, or multi-hexamers, with subunits of molecular weight (MW) ~75,000, while molluscan haemocyanins are cylindrical oligomers with subunits of molecular weight ~400,000 (refs 20–25).

The oxygen-binding centres of haemocyanins each contain a pair of copper ions and have been the subject of extensive spectroscopic analysis. It is generally believed that the oxygen-binding centres of arthropodan and molluscan haemocyanins are quite similar[25–27]. Furthermore, the spectrum of the binuclear copper-containing oxytyrosinase is remarkably similar to that of oxyhaemocyanin[28–30]. Other proteins are known also to contain binuclear copper sites, such as ascorbate oxidase, laccase and ceruloplasmin[31–33], so knowledge of the binuclear copper site in arthropodan haemocyanins may lead to a better understanding not only of these sites in molluscan haemocyanins but also of those in several enzymes.

We have investigated the haemocyanin of the spiny lobster *Panulirus interruptus*, an arthropod. It is one of the smallest haemocyanins known, but still has a molecular weight of ~450,000 (ref. 34). It is a hexamer, each subunit containing one copper pair required for its oxygen transport function[25,35]. The oxygen-binding properties of *P. interruptus* haemocyanin[34–37] exhibit a distinct cooperativity, as do those of all haemocyanins[25].

As is common among arthropodan haemocyanins[25], *P. interruptus* haemocyanin displays subunit heterogeneity[13]. At least three types of subunits occur, designated a, b and c (formerly called '94K', '90K' and '80K', see below). The crystals used for the X-ray studies were grown from native *P. interruptus* haemocyanin. When the crystals were dissolved it was found that they only contain a and b subunits, in roughly equal amounts (J. P. van Eerd, unpublished results). Although immunological data suggest that these two types of subunits differ in amino acid sequence by ~18% (ref. 38), their N-terminal sequences are very similar[13] and no problems were encountered in interpreting the electron density distribution. The polypeptide chain could be followed very easily in a 3.2 Å electron density map of a molecule with a molecular weight of nearly half a million even though for half of the reflections no heavy atom phase information was available.

Amino acid sequence

The amino acid sequence determination was carried out on subunit a of *P. interruptus* haemocyanin. This subunit was formerly called '94K' after its apparent molecular weight (94,000) on SDS-polyacrylamide gels[13]. This nomenclature has now been abandoned, because, as shown below, the number of residues appears to be ~660, indicating a subunit molecular weight of ~75,000. This agrees with earlier gel filtration and sedimentation equilibrium studies by Kuiper *et al.*[34].

As a starting point for the primary structure determination, limited proteolysis of subunit a was carried out[14]. This generated an 18K fragment, derived from the N-terminus of the subunit, a 71K fragment from the C-terminus and a glycopeptide from in between these two fragments. The apparent molecular weights of these fragments were determined from their mobilities on SDS-polyacrylamide gels. For the N-terminal fragment the molecular weight determined agrees well with its length of 160 residues. The 71K fragment, however, has ony ~485 residues, and its anomalous behaviour on SDS gels is not understood.

The tryptic fragments were subjected to further digestions, followed by amino acid sequence analysis. Parts of the amino acid sequence of *P. interruptus* haemocyanin, including previously published sequences[13–17], are presented in Fig. 1.

Crystal structure determination

The monoclinic crystals, studied at *p*H 4.5, contained the complete 450,000-MW hexamer in the asymmetrical unit[34,39]. The crystal structure of *P. interruptus* haemocyanin at 5.0 Å has been reported elsewhere[39]. These studies revealed the shape and the 32 point group symmetry of the hexamer, the orientation of the molecular symmetry axes and the molecular centre. No heavy-atom derivative information was used beyond 4 Å. The procedure for obtaining the ultimate 3.2-Å electron density map was as follows.

(1) The 36 heavy-atom sites of the K_2PtCl_4 derivative and the 70 *o*-mercury phenol positions known from the 5-Å studies were optimized, by the lack of closure-error refinement method[40], against the 4-Å native and derivative data sets, followed by phase[41] and electron density calculations.

(2) The resultant 4.0-Å multi-isomorphous replacement electron density map was subjected to six cycles of Bricogne's molecular replacement program for phase improvement[42]. The

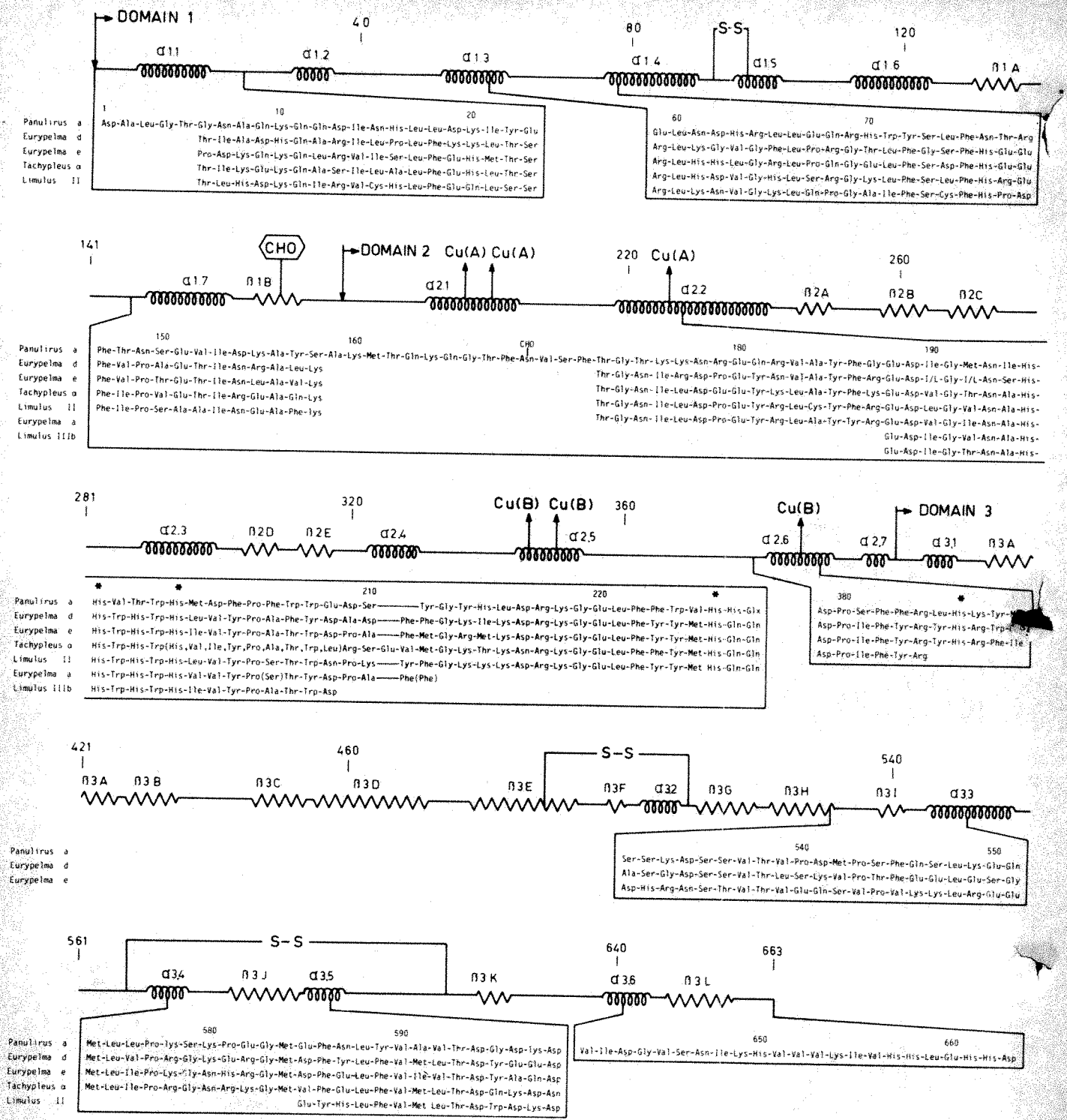


Fig. 1 Secondary structure and partial amino acid sequence of *Panulirus interruptus* haemocyanin subunit a, together with sequence information for several other arthropodan haemocyanins. The carbohydrate moiety (CHO), as well as the copper ligands (vertical arrows and asterisks) are indicated. The starting points of the different domains are indicated by the horizontal arrows. The α-helices are indicated by the coiled line, and the β-strands by the zig-zag. A two-character identifier is assigned to each α-helix and β-strand: the first character is the domain number, the second gives the position of a secondary structure element within its domain (for example, α 3.4 is the fourth helix in the third domain; β 2C is the third β-strand in the second domain). The amino acid numbering scheme must be considered as tentative, as the *P. interruptus* sequence is not yet complete and the number of residues cannot be established with certainty from the X-ray analysis at the present resolution. For the regions shown, the *P. interruptus* sequence data fit well into the electron density map. The disulphide bridges, indicated by S—S, are based on the electron density map, supported by the sequence information that residue 92 is a cysteine. The first and last three residues of the polypeptide chain are invisible in the map. Weak, but continuous, density occurs for residues 562–566 and 603–613. These weaker densities may be due to different courses of the polypeptide chain in the a and b subunit. Otherwise the entire polypeptide chain is well defined, including numerous side chains. As the N-terminal as well as the C-terminal fragments, of which the amino acid sequences are shown, could be unambiguously placed in the electron density map, as shown, there is no doubt that the complete polypeptide chain has been traced. For selected regions of the chain, apparently related amino acid sequences of other arthropodan haemocyanins are presented. The sequences shown are: *Eurypelma californicum* subunits d (ref. 12), e (ref. 11) and a (ref. 19); *Tachypleus tridentatus* chain α (ref. 8); and *Limulus polyphemus* subunits II (refs 9 and 10, and E. Yokota and A. F. Riggs, personal communication) and IIIb (ref. 19).

shifts in the phases (Table 1) and the improvement in the electron density map were considerable. The copper positions became evident at this stage[43].

(3) The phases obtained from Fourier inversion of the averaged electron density map were added to the known phases in thin shells of increasing resolution, containing 1,900–3,300 reflections. After one phase extension step, three cycles of density averaging and phase improvement were performed at constant resolution. Next, a new shell of reflections with unknown phases was added, and the procedure repeated. After the tenth and last extension step, 11 phase improvement cycles were performed.

The 3.2-Å data set contained 63,842 amplitudes, of which 31,121 had no heavy-atom derivative phase information. The statistics of the procedure are given in Table 1.

Polypeptide chain folding

The resultant 3.2-Å averaged electron density map proved to be of excellent quality (Fig. 2). It appeared possible to trace the complete polypeptide chain, to indicate disulphide bridges and to position several peptides for which the amino acid sequence was known (Fig. 1). The copper ions were by far the most prominent features of the map. The carbohydrate moiety[14,44] was clearly visible, as shown in Fig. 2.

A *P. interruptus* haemocyanin subunit folds its ~660 residues into an irregular ellipsoid of dimensions 48×55×80 Å, in accordance with electron microscopic data[20,22]. There are three distinct domains (Figs 3, 4). The first domain comprises 177 residues and is virtually all helical (Figs 1, 3*d*, 4*a*). A disulphide bridge occurs between residues 92 and 97. The last β-strand of the first domain is incorporated into a small, three-stranded β-structure together with two strands provided by one of the long loops of the third domain (Fig. 3*c*, *d*). This β-strand of domain 1 contains the single carbohydrate moiety of *P. interruptus* haemocyanin, which is covalently attached to residue Asn

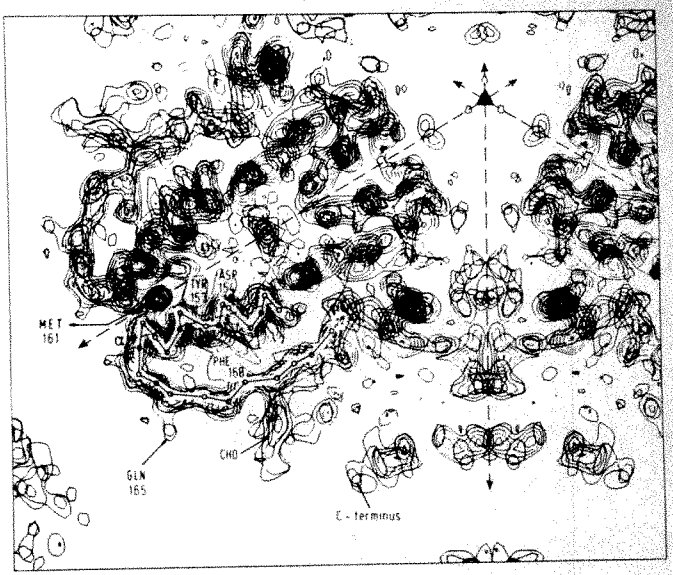


Fig. 2 Part of the ultimate, averaged, 3.2-Å electron density map. Five sections are shown through the centre of the hexamer, viewed along the local 3-fold axis of symmetry (▲). The thickness of the slab shown is 6 Å. As the hexamer obeys 32 point group symmetry[39], three local 2-fold symmetry axes occur in the central section, as indicated. For one subunit α-carbon positions in α-helix 1.7 and β-strand 1 B are indicated. CHO, the carbohydrate moiety. Some well defined side chains are labelled, and the same densities can be seen in the symmetry-related subunit. The course of the polypeptide chain can be followed without any difficulty, and numerous side chains have a well developed density. In the map actually used for chain tracing and peptide fitting, twice as many sections were used, at distances of 0.75 Å from each other.

Table 1 3.2-Å phase statistics

Resolution limits (Å)	No. of reflections	*R*-factor* (%)	Figure-of-merit†
20.0–8.94	3,261	18.5	0.75
8.94–6.32	6,180	15.4	0.82
6.32–5.16	7,646	18.3	0.81
5.16–4.47	9,004	20.6	0.78
4.47–4.00	9,696	23.6	0.76
4.00–3.65	10,113	26.7	0.74
3.65–3.38	9,960	30.1	0.71
3.38–3.16	7,982	35.7	0.65
Total	63,842	23.5	0.75

For the double isomorphous replacement phases, out to 4 Å, the average figure-of-merit (as defined by Blow and Crick[41]) was 0.57 for 32,721 reflections. After six cycles of phase improvement by density averaging[42], the average figure-of-merit, as defined by Sim[66], was 0.76 with an average phase change of 60.4° with respect to the isomorphous replacement phases. The table provides statistics as a function of resolution after the phase extension (as described in the text) out to 3.2 Å. Molecular replacement programs were provided by Dr G. Bricogne and rewritten in FORTRAN 77 for the Cyber 170/760 of the Computer Centre of the University of Groningen, where all computations were performed.

* $$R = 100 \times \frac{\sum (|F_{P,calc}| - |F_{P,obs}|)}{\sum |F_{P,calc}|}$$

with

$$|F_{P,calc}| = k \exp\left[-B\left(\frac{\sin\theta}{\lambda}\right)^2\right]|F_{P,inv}|$$

where $|F_{P,inv}|$ is the structure amplitude obtained by Fourier inversion of the averaged electron density map; k and B are relative scaling and temperature factors obtained by a relative Wilson plot.

† The average phase difference between the phases after six cycles of molecular replacement at 4 Å resolution and the phases obtained by the multiple isomorphous replacement method.

169 (ref. 14). It appears that the carbohydrate moiety protrudes into a solvent region and does not, except for the first sugar group, make contacts with amino acid side chains. In this respect, the *P. interruptus* haemocyanin sugar chain is distinctly different from immunoglobulins[45], but similar to some carbohydrate chains in haemagglutinin[46] and neuraminidase[47].

The second domain, comprising residues 177–400, is also largely helical, but has a few β-strands (Figs 1, 3*e*, 4*b*). Four of these strands, 2B and 2C, and 2D and 2E, form antiparallel ββ-units. The oxygen-binding site is located in the centre of this globular domain, in between four helices. This site will be described in more detail later.

The third and largest domain consists of residues 400–663. From the central region of this domain extend long loops (Figs 3*f*, 4*c*) which appear to function as arms holding the three domains together (Fig. 3*c*). The central part of this domain is an antiparallel β-barrel consisting of seven strands. The electron density map indicates two disulphide bridges in this domain (Figs 1, 3*f*, 4*c*), giving a total of three disulphide bridges in each haemocyanin subunit.

Primary structure comparisons (Fig. 1) show that all three domains contain regions of similar sequence between haemocyanins of different organisms, which suggests strongly that the three-domain structure is a common feature of all arthropodan haemocyanins.

Figure 3*a* shows a stereoview of the hexamer and Fig. 3*b* the 'upper' trimer. In the hexamer, all three domains are involved in inter-subunit contacts. For instance, helix 1.7 is in contact with the same helix in a subunit related by a 2-fold axis (see Fig. 2). Helices 2.3 from three subunits interact with each other around the molecular 3-fold axis (Fig. 3*b*), and helix 3.6 is close to helix 1.3 of another subunit. As in haemoglobin[1,3] and haemerythrin[5–7], the contacts are all mediated by interactions between side chains—no hydrogen bonding interactions between backbone atoms occur.

The oxygen-binding site

The crystals that were used in the X-ray analysis were colourless, which suggests that we have determined the structure of the

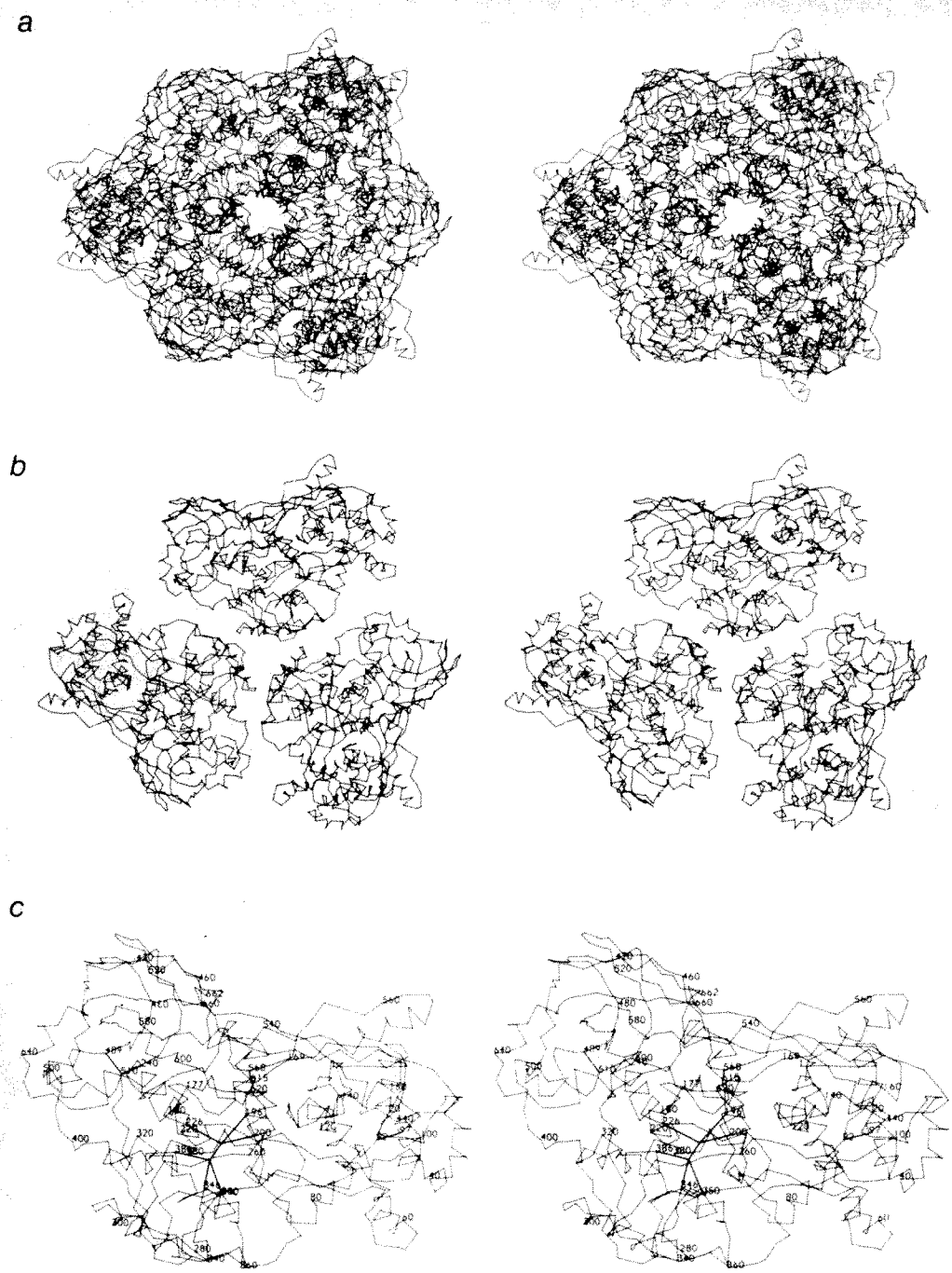


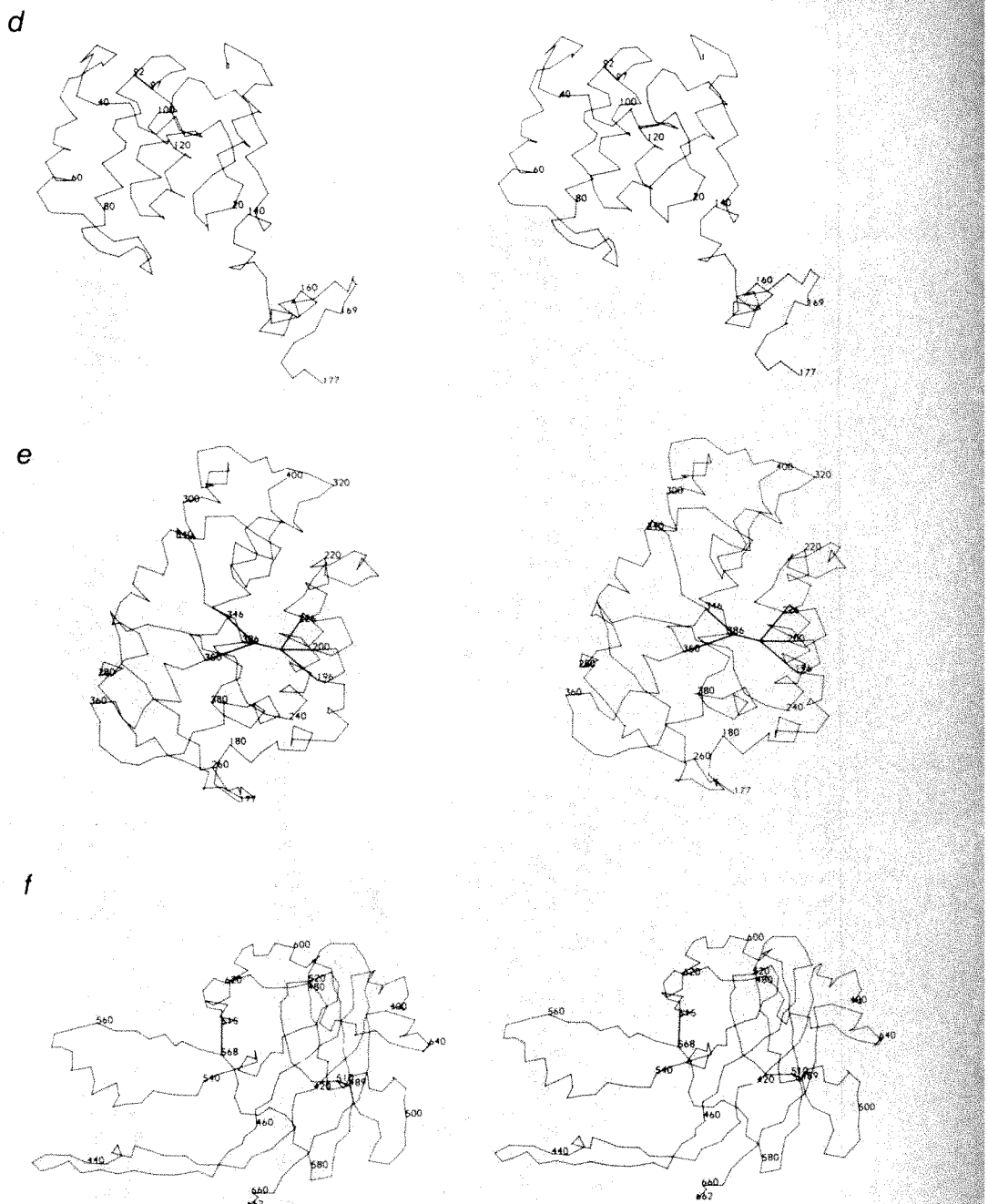
Fig. 3 The three-dimensional structure of *P. interruptus* haemocyanin in stereoscopic projection. Coordinates of the α-carbon, γ-sulphur and copper atoms were directly measured from a 3.2-Å electron density map, which was plotted on transparent sheets at a scale of 1 cm = 4 Å. Although the general course of the chain could be followed without difficulty, α-carbon coordinates of certain loops with less well developed side chains may be less accurate than that of other regions. ***a***, The complete hexamer, with a total of 3,978 residues, viewed along the molecular 3-fold axis. The three molecular 2-fold axes of this molecule with point group 32 (ref. 39) are in the plane of the paper. The hexamer can be described as two trimers, related by 2-fold axes and stacked face-to-face upon each other. ***b***, A view along the local 3-fold axis of the 'upper' trimer of *a*. ***c***, A view along the local 3-fold axis of one subunit only, with the same residues labelled as in *d–f*. Residue 169 contains the carbohydrate moiety. Residue 177 is the end of the first domain, and number 400 the end of the second domain. ***d***, The first domain only, in a view which gives a clear picture of the course of the polypeptide chain. The position of the first three residues is arbitrary as no density was visible in the electron density map. One disulphide bridge is indi-

deoxygenated form of haemocyanin. This is consistent with the low oxygen affinity of *P. interruptus* haemocyanin at low pH[48], and the absence of an absorption band near 340 nm in single-crystal UV spectroscopy (M. Vincent, unpublished data). However, we cannot rule out completely that the structure we have determined is of Met, or half-Met, haemocyanin.

It is clear from the 3.2-Å electron density map that each copper ion has three protein ligands. No evidence for a bridging protein ligand is found. One of the pair of copper ions, Cu(A), is surrounded by residues 196, 200 and 226; the other, Cu(B), is surrounded by residues 346, 350 and 386. Two of the ligands of Cu(A) come from successive turns of helix 2.1, and the equivalent two ligands of Cu(B) come from successive turns of helix 2.5. The third ligand of each copper ion also comes from a helix. The six copper ligands are thus provided by four helices. This is reminiscent of the situation in haemerythrins[5–7], but the topologies of the helices surrounding the oxygen-binding sites are different in these two classes of oxygen-binding proteins. Antiparallel helices both providing ligands for metal ions are also found in Fe-superoxide dismutase[49,50].

All six copper ligands in haemocyanin are almost certainly histidines. For four of the ligands (residues 196, 200, 226 and 386) this is clear from the fit of the *Panulirus* haemocyanin amino acid sequence with the electron density map. All other sequenced haemocyanins also have histidine residues at these positions, and very similar sequences in the surrounding regions (Fig. 1). The two remaining Cu(B) ligands have not been sequenced yet for *P. interruptus* haemocyanin. These two ligands must have the sequence L_1-X-X-X-L_2 according to the electron density map, with L_2 approximately 36 residues away from the third ligand. In the sequences of *Tachypleus* haemocyanin[8], and of two *Eurypelma* haemocyanin subunits[11,12] the arrangement His-X-X-X-His is observed precisely at the expected position. As the amino acid sequences of these haemocyanins are clearly related to that of *P. interruptus* haemocyanin in other regions (Fig. 1), it is justified to conclude that in arthropodan

cated by a virtual bond between the α-carbon atom of residues 92 and 97. Up to residue 155, this domain is quite globular, but residues 156–177 form an 'appendix' comprising helix 1.7 and β-strand 1 B. Helix 1.7 forms contacts via a 2-fold axis with another subunit as shown in Fig. 2. Strand 1 B contains the carbohydrate chain at position 169, and forms a β-structure with strands 3B and 3C of the long extended loop, 440–480, of the third domain (*c*). *e*, The second domain in a view allowing an easy following of the chain. The positions of the copper atoms are indicated and connected to the α-carbon atoms of the liganding residues. This domain is clearly globular and is mainly helical. The six protein ligands of the two copper ions are all histidines (see text). It should be emphasized that, at the present resolution, we cannot exclude the possibility of a small exogenous bridging ligand, such as a μ-oxo atom. *f*, The third domain oriented in such a manner as to highlight the two long excursions of the chain. Two probable disulphide bridges are indicated. It is quite obvious that this domain is far from globular. The long loops which extend from the central β-barrel make intimate contacts with the two other domains (*c*). The position of the last three residues is arbitrary as only weak density was present in the electron density map for these residues.



haemocyanins the two copper ions are liganded by six histidines. The involvement of the histidine cluster at position 195–200 in copper binding has been correctly suggested by Schneider *et al.*[19] and Yokata *et al.*[9]. That each copper atom has three ligands agrees well with the suggested model of the oxygen-binding site for deoxyhaemocyanin by Brown *et al.*[51] and Larrabee and Spiro[52].

The distance between the two electron density maxima representing the copper ions is 3.8 Å, with an error of ~0.4 Å. This corresponds well with the values derived from EXAFS experiments: 3.39 Å[51] for deoxyhaemocyanin; 3.67 Å[51] and 3.55 Å[53] for oxyhaemocyanin. The copper-binding centres in the subunits of a haemocyanin trimer (Fig. 3*b*) are separated by distances of 46 Å. The copper centres in one 'upper' subunit are separated from those in the three 'lower' subunits by distances of 47, 54 and 68 Å. Cooperative interactions between subunits thus must operate over quite large distances in *P. interruptus* haemocyanin.

Binuclear copper sites

No other three-dimensional structures are yet available for proteins with binuclear copper sites. However, the complete amino acid sequence of *Neurospora crassa* tyrosinase[54], a nearly complete sequence of human ceruloplasmin[32,33] and small fragments of the sequence of laccase[55,56]—all of which have binuclear copper sites—are known. Does the sequence His-X-X-His, which provides four of the ligands for each haemocyanin binuclear copper centre, occur in these possibly related proteins?

In tyrosinase this sequence does indeed occur: His 277 and His 281 are separated by three residues and are therefore possible copper ligands. Two other histidine residues in tyrosinase have been implicated by photo-inactivation as copper ligands: His 188 and His 193 (ref. 54). These occur in a sequence resembling that which provides two of the copper ligands in haemocyanin[9], but they are separated by four, not three, residues. This suggests that there are variations in the structure

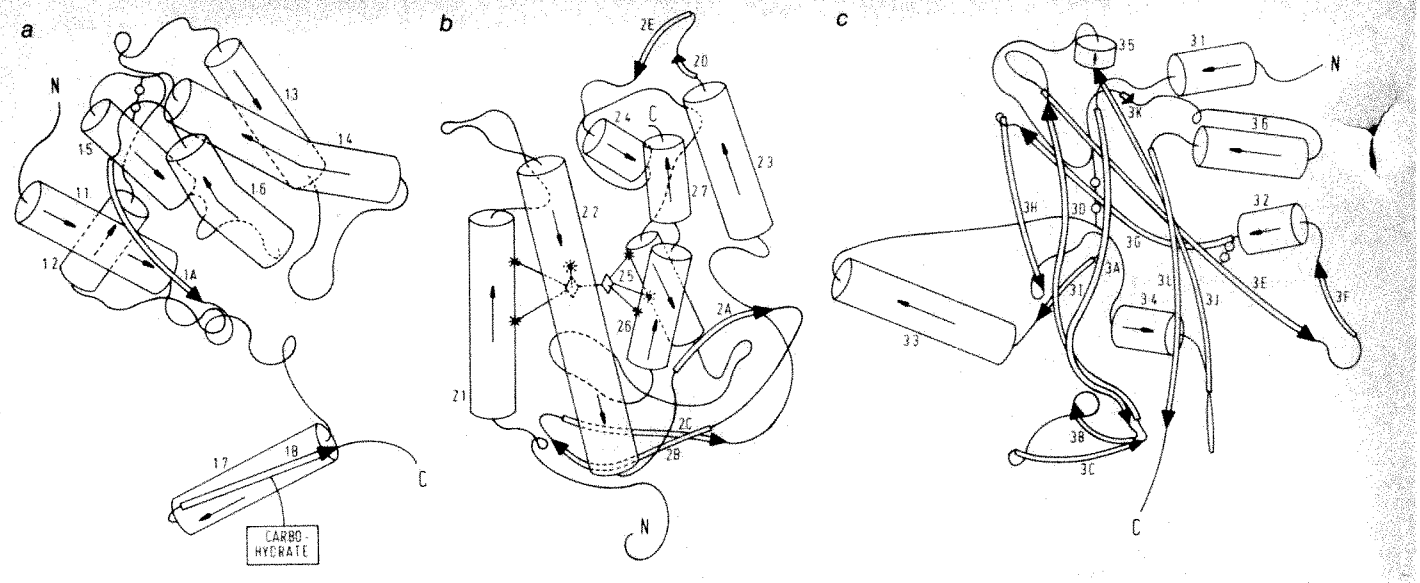


Fig. 4 The three domains of a subunit of *P. interruptus* haemocyanin depicted schematically, viewed from the same direction. ***a***, Domain 1: Helices are indicated by cylinders and β-strands by arrows. Secondary structure elements are indicated as in Fig. 1. A disulphide bridge is shown as —○—○—. The 'appendix' formed by helix 1.7 and β-strand 1B is evident. This β-strand contains the carbohydrate moiety. ***b***, Domain 2: The copper ions, which are located in the centre of this domain, are indicated by diamonds. All six ligands of the copper ions can clearly be seen to be provided by residues belonging to α-helices. It may be noted that the first ligand of Cu(B), residue 346 of helix 2.5, is situated at the very N-terminal turn of this helix. This is quite different from the first ligand of Cu(A), which resides in the middle of helix 2.1, and from the Fe-ligands in haemerythrin, which are also provided by residues from central parts of helices. ***c***, Domain 3: Two probable disulphide bridges are indicated by —○—○—. In this view, one long loop, comprising α3.3, extends to the left while another one, connecting β-strands 3B and 3C, extends to the back of this picture. (Drawings by A. Volbeda.)

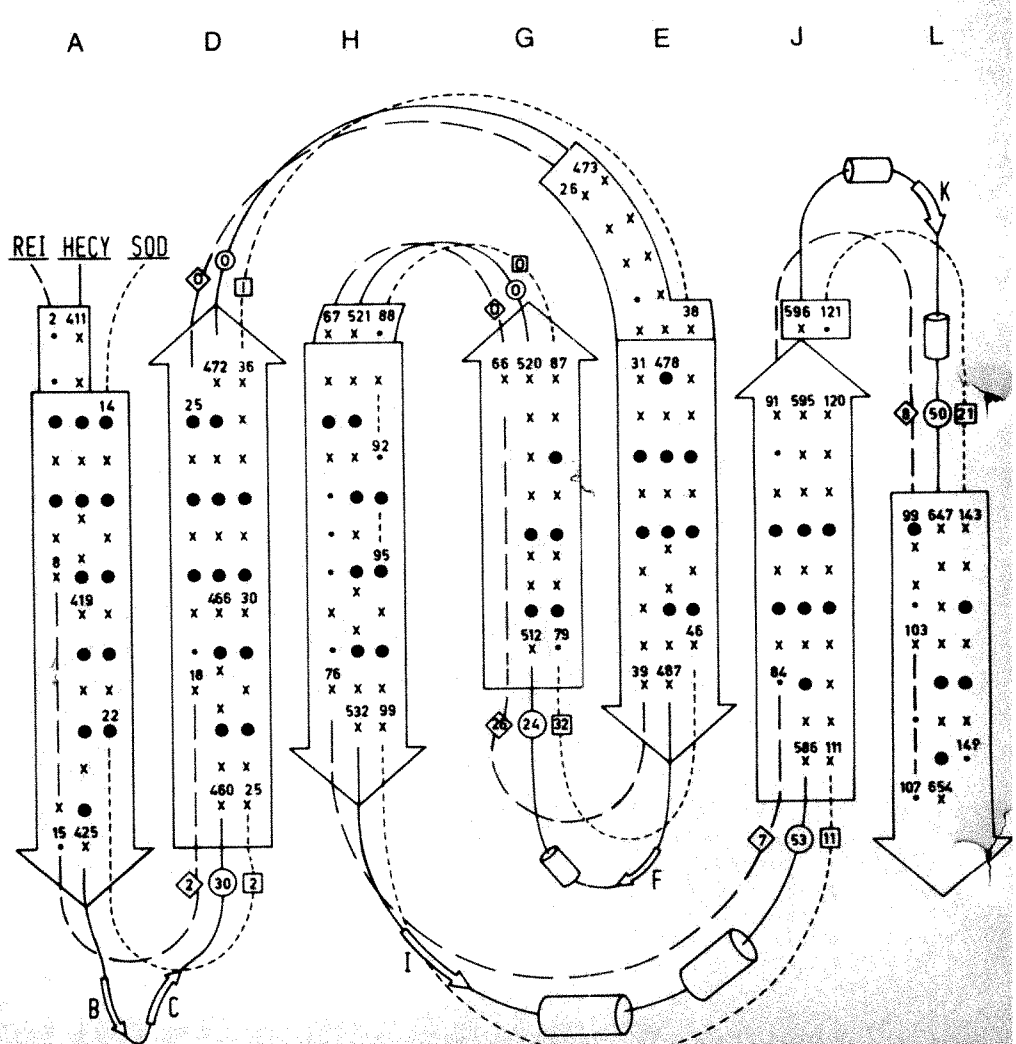


Fig. 5 Alignment of the equivalent amino acid residues in the β-barrels of Cu, Zn superoxide dismutase (SOD)[62], immunoglobulin REI (REI)[63] and the third domain of *P. interruptus* haemocyanin (HECY). The large arrows represent the seven β-strands which form the major part of the barrel. Equivalent α-carbon atoms which superimpose within 3.2 Å with haemocyanin are shown as large dots (●) and crosses (×). Equivalent α-carbon atoms deviating more than 3.2 Å but less than 4.0 Å are indicated by small dots (·). ● Indicate residues that point their side chains into the interior of the β-barrel. Residues marked by · or × do not point towards the interior. Strands G, E, J and L (haemocyanin numbering as in Figs 1 and 4) form one side of the barrel, strands A, D and H the other side. The lengths of the loops connecting the strands are also indicated: those of Cu, Zn superoxide dismutase in squares; those of immunoglobulin REI in circles; and those of haemocyanin in diamonds. (Drawing by A. Volbeda.) It appears that the third domain of haemocyanin has by far the largest loops. The loop connecting strands D and E is very similar in immunoglobulin REI and haemocyanin. The short loop connecting strands G and H is the same in all three cases. The three β-structures appear to resemble each other remarkably well (see text). For Cu, Zn superoxide dismutase and immunoglobulin REI, the coordinate sets 2SOD and 1REI from the Protein Data Bank[67] have been used for the superposition studies.

of the polypeptide chains surrounding these binuclear copper sites in different molecules, although spectroscopic data suggest that the copper environments are actually very similar[28–30].

In ceruloplasmin two pairs of histidines separated by three residues occur[32,33], and in the short sequence known of laccase[55] one such pair has been found. On the basis of the haemocyanin structure, these histidines may be binuclear copper ligands, although for ceruloplasmin other models have been suggested[32,33].

Molluscan haemocyanin structure

Knowledge of the oxygen-binding domain of arthropodan haemocyanins suggests a model for the molluscan haemocyanin structure. The subunit molecular weight of molluscan haemocyanins is ~400,000, with ~50,000 per binuclear copper site[20–22,25]. The arthropodan oxygen-binding domain, with ~223 residues, has a molecular weight of ~25,000. Spectroscopic evidence suggests that the oxygen-binding centres of molluscan and arthropodan haemocyanins are very similar[25–27]. The basic 50,000-MW unit in molluscan haemocyanins may therefore consist of an oxygen-binding domain similar to the one in *P. interruptus* haemocyanin, and a second domain which might correspond to the first or third *P. interruptus* haemocyanin domain. The two domains would be in quite intimate contact, forming a globular unit, while an array of eight such units makes up a complete molluscan haemocyanin subunit[57–59]. Similar relationships between the two haemocyanin classes have been suggested, on different grounds, by Van Holde[60].

Convergent evolution

The core of the third haemocyanin domain consists of a seven-stranded, antiparallel β-barrel. Surprisingly, the topology of this core is identical to that of the β-barrels in immunoglobulins and in Cu, Zn superoxide dismutase (Fig. 5)[61–63]. After optimal superposition of ~60 equivalent C^{α}-positions, the three barrels deviate mutually by less than 2.5 Å (r.m.s.).

However, when the available amino acid sequence for the β-barrel region of *P. interruptus* haemocyanin is compared with the sequences of the other β-barrels, hardly any similarities are observed. This is also the case when the β-barrel sequences of immunoglobulins and Cu, Zn superoxide dismutase are compared[61]. This β-barrel structure is probably an inherently stable structure, which allows great variability in length and folding patterns of the loops connecting the strands. The appearance of such β-barrels in domains with totally different functions suggests that the structural relationship is the result of convergent evolution.

Conclusion

The combination of X-ray diffraction and amino acid sequencing techniques described here has allowed the elucidation of the architecture of *P. interruptus* haemocyanin, which is probably common to all arthropodan haemocyanins. Furthermore, a detailed view has been obtained of the oxygen-binding site, which may share many features with the binuclear copper sites found in molluscan haemocyanins and several other proteins. Important questions remain to be answered, however, such as the nature of the contacts between the subunits of this hexameric protein, the structural changes that occur on oxygenation (fortunately, the X-ray analysis of an oxyhaemocyanin is under way[64,65]), and the relationship between the structures of arthropodan and molluscan haemocyanins. Once these questions are settled, then a complete picture will have been obtained of the three, or perhaps four, solutions nature has found for reversible oxygen transport in living organisms.

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Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule

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The ability of fibronectin to bind cells can be accounted for by the tetrapeptide L-arginyl-glycyl-L-aspartyl-L-serine, a sequence which is part of the cell attachment domain of fibronectin and present in at least five other proteins. This tetrapeptide may constitute a cellular recognition determinant common to several proteins.

FIBRONECTIN is a well characterized extracellular, structural glycoprotein which interacts strongly with other extracellular matrix molecules and which causes the attachment and spreading of most cells when presented to them as an insoluble substrate. This latter property is of considerable interest because an understanding of how cells interact with their surroundings may lead to an understanding of differentiation and of cell migration, for example, that which occurs during embryonic development or in tumour metastasis.

By using a monoclonal antibody which inhibits cell attachment, we have recently isolated, as a peptic fragment of molecular weight (MW) 11,500 (11.5K), the domain within the fibronectin molecule which endows it with the ability to interact with the cell surface of fibroblasts[1,2], and we have determined the complete amino acid sequence of this fragment[2]. This domain contained all of the cell attachment activity to be found among the proteolytic fragments of the fibronectin molecule[1]. By using synthetic peptides modelled from the sequence of this domain, we have described a 30-amino acid synthetic peptide which carries the cell attachment-promoting activity[3]. By using smaller synthetic peptides, we have now localized this function of the fibronectin molecule to a sequence of only four of these amino acids and we have shown that the same tetrapeptide is present in some other proteins which may interact with cells, including fibrinogen[4], a surface protein of *Escherichia coli*[5], and a Sindbis virus coat protein[6].

Cell attachment-promoting activity

The 30-amino acid synthetic peptide described previously placed the cell attachment site within the COOH-terminal quarter of the 108-amino acid cell attachment domain[3]. At that time, we predicted, by the method of Rose[7], that the cell attachment-promoting activity of the fibronectin molecule would probably be localized close to a hydrophilic stretch of amino acids near the NH_2-terminus of that peptide. Further analysis of the cell attachment domain, using methods for predicting secondary structure described elsewhere[8,9], showed that the group of residues boxed in Fig. 1 may be a β-turn which probably forms a hydrophilic loop at the surface of the molecule and is thus available to interact with the cell surface. To test whether these amino acids were involved in the cell attachment activity of the peptide, we obtained several synthetic peptides (Fig. 2*a*), coupled them to protein-coated plastic and tested each for cell attachment-promoting activity (Fig. 2*b*). All the peptides that promoted the attachment of rat kidney fibroblasts (NRK cells) contained the sequence Arg-Gly-Asp-Ser (or Arg-Gly-Asp-Cys) (Fig. 2*b*). The activity of the peptides decreased somewhat with size, perhaps due to a decrease in the stability of their conformation or to a relative inaccessibility on the substrate. The tetrapeptide, Arg-Gly-Asp-Ser, itself was active when immobilized on Sepharose beads at the end of a C_6 spacing arm (Fig. 3). Poor accessibility due to its small size or low efficiency of the coupling methods used for this peptide may account for the fact that we could not demonstrate activity in this peptide after coupling to protein-coated plastic.

Analysis of the data in Fig. 2 indicates that the serine residue of the tetrapeptide sequence can be replaced by a chemically similar cysteine residue without substantial loss of the activity (peptide IVA1a in Fig. 2). The activity was lost, however, when the arginine or aspartic acid residues were selectively deleted (Fig. 2) or if the synthetic peptides were cleaved at the arginine with trypsin (data not shown). Also, substitution of the glycine with the bulky valine residue (peptide RVDSPAC in Fig. 2) abolished the activity. Moreover, as can be seen in Fig. 4*a*, substitution of aspartic acid with asparagine greatly diminished the activity of the resulting tetrapeptide. We conclude from these results and from the data presented below that the Arg-Gly-Asp sequence must be maintained to preserve activity, while some variation at the position occupied by the serine residue is compatible with activity.

Inhibition of cell attachment

We were unable to inhibit the attachment of cells to fibronectin-coated surfaces with soluble fibronectin, nor could we inhibit such attachment with obtainable concentrations of the 30-amino acid, cell attachment-promoting synthetic peptide[3]. A possible explanation for this lack of inhibition is that the affinity of a

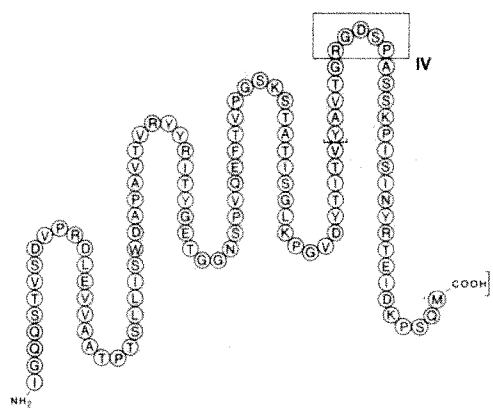


Fig. 1 Prediction of the secondary structure of the 11.5 K cell attachment domain of fibronectin. The methods of Chou and Fassman[8] and Kyte and Doolittle[9] were used to predict the probable secondary conformation of the cell attachment domain of fibronectin based solely on the primary structure[2]. The peptide consists of a series of potential β-turns, the most hydrophilic of which (boxed) is shown here to contain the cell attachment signal. Peptide IV, which was shown previously to contain the cell attachment activity[3], is enclosed in brackets. The one-letter code used to denote each amino acid is: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.

single fibronectin molecule for the cell surface is so low that very high concentrations of soluble fibronectin or its fragments would be needed to overcome the cooperative interaction of many immobilized fibronectin molecules with cells. That this is the case has been suggested by the work of Grinnell and colleagues[10,11] and by that of Hughes *et al.*[12].

The small peptides, in contrast to fibronectin and its larger fragments, are highly soluble, which makes it possible to test them at millimolar concentrations for inhibition of cell attachment. For these tests we chose peptides which did not have a cysteine residue to avoid possible binding of the peptide to the substrate by disulphide exchange. We found that the Gly-Arg-Gly-Asp-Ser-Pro and Arg-Gly-Asp-Ser peptides did indeed interfere with the attachment of NRK cells to fibronectin-coated substrates (Fig. 4*a*). (In experiments where they were compared directly, the Gly-Arg-Gly-Asp-Ser-Pro peptide promoted cell attachment to surfaces coated with it to the same extent as did Gly-Arg-Gly-Asp-Ser-Cys.) The inhibition by the peptides occurred, in a dose-dependent manner, at peptide concentrations $>1\times10^{-4}$ M, (Fig. 4*b*). An affinity constant for the cell surface of 3×10^{-4} M and 6×10^{-4} M for the hexapeptide and tetrapeptide, respectively, was obtained using the method for estimating antibody affinity described by Müller[13]. Peptides not containing the Arg-Gly-Asp-Ser sequence showed no inhibition of cell attachment to fibronectin, indicating that the inhibition is specific and that inclusion of peptides in the cell attachment assay at these concentrations had no adverse effects on the cells. Furthermore, the attachment of cells to concanavalin A (Sigma) remained unaffected by the Arg-Gly-Asp-Ser-containing peptides (data not shown).

The tetrapeptide in other proteins

Having established the importance of the Arg-Gly-Asp-Ser sequence in fibronectin, we looked for this tetrapeptide in other proteins by searching the library of sequences compiled by the National Biomedical Research Foundation (George Washington University Medical Center, Washington, D.C.). This search revealed five proteins having the tetrapeptide (Table 1). Taking into account the frequency at which each of these four amino acids occurs in proteins and the 351,138 tetrapeptides examined from the sequence library, six such sequences would have been expected.

Of the five proteins having the active tetrapeptide sequence, fibrin(ogen) was of most immediate interest because of its demonstrated interaction with fibronectin[14] and the platelet surface[15,16]. Therefore, we tested a synthetic nonapeptide modelled on residues 568–580 of the α-chain of fibrinogen and found that it also promoted cell attachment Table 1 and Fig. 5). Attempts to demonstrate the attachment of our test cells to intact fibrinogen or fibrin, however, have not given clear-cut results. In accordance with earlier results[17], no attachment of fibroblastic cells to fibrinogen insolubilized on plastic could be demonstrated. Attachment of cells to the fibrinogen or fibrin polypeptides, separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose as described elsewhere[18], gave only weak or, at times, no attachment to the separated α-chain (data not shown). In contrast, the activity of fibronectin is readily detectable in both conditions. It may be that the cell attachment site in fibrinogen is cryptic or destroyed during the assay procedures, or that the surrounding sequences

a

Code	Sequence	Concentration for 50% cell attachment
Fibronectin		0.10 nmol ml^{-1}
IV	Y A V T G R G D S P A S S K P I S I N Y R T E I D K P S Q M (C)	0.25
IVA	V T G R G D S P A S S K P I (C)	1.6
IVB	S I N Y R T E I D K P S Q M (C)	>50.0
IVA1	V T G R G D S P A (C)	2.5
IVA2	S P A S S K P I S (C)	>50.0
IVA1a	V T G R G D (C)	10.0
IVA1b	G R G D S (C)	3.0
IVA1c	R G D S P A (C)	6.0
RVDS	R V D S P A (C)	>50.0

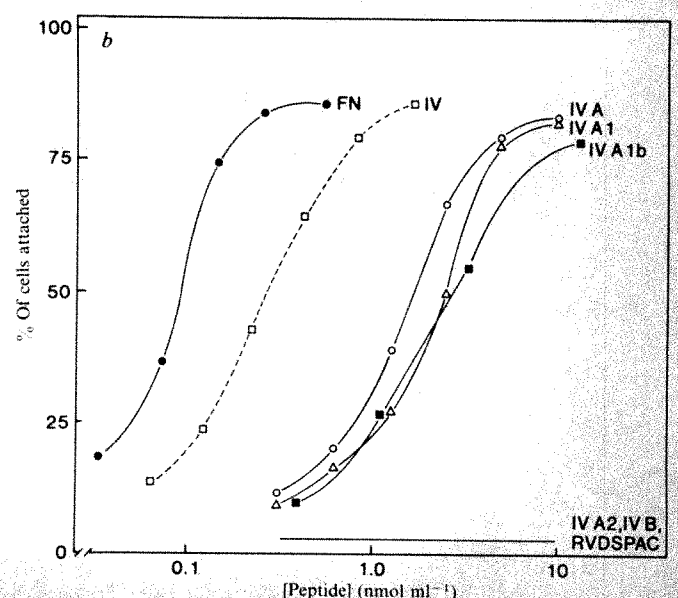


Fig. 2 Attachment of NRK cells to immobilized fibronectin and synthetic fibronectin peptides. ***a***, Peptides were synthesized by Peninsula Laboratories (Belmont, California) according to our specifications, to include a COOH-terminal cysteine (C). The activity of each peptide is indicated on the right of the figure by the concentration necessary to achieve 50% maximal attachment in the assay described below. ***b***, The synthetic peptides were assayed for their ability to promote the attachment of NRK cells by first attaching the peptides via the heterobifunctional cross-linker SPDP (*N*-succinimidyl-3-(2-pyridyldithio) propionate; Sigma) to rabbit IgG which had been immobilized on polystyrene[3]. By using radiolabelled peptides or antibodies to the larger peptides, we have determined that coupling of the peptides using this method is more than 85% complete (data not shown). Peptides not containing a cysteine are not included in the figure as the methods used to immobilize them appeared to be less efficient (see text). The attachment assay was carried out as described[35] using freshly trypsinized NRK cells. After incubation for 1 h at 37°C, those cells which had attached were fixed, stained and quantitated using either an Artek cell counter or a vertical pathway spectrophotometer. In all cases, maximum attachment was ~80–90% of the cells plated. The titration curves for whole fibronectin (FN) and peptide IV (ref. 3) are shown here for comparison.

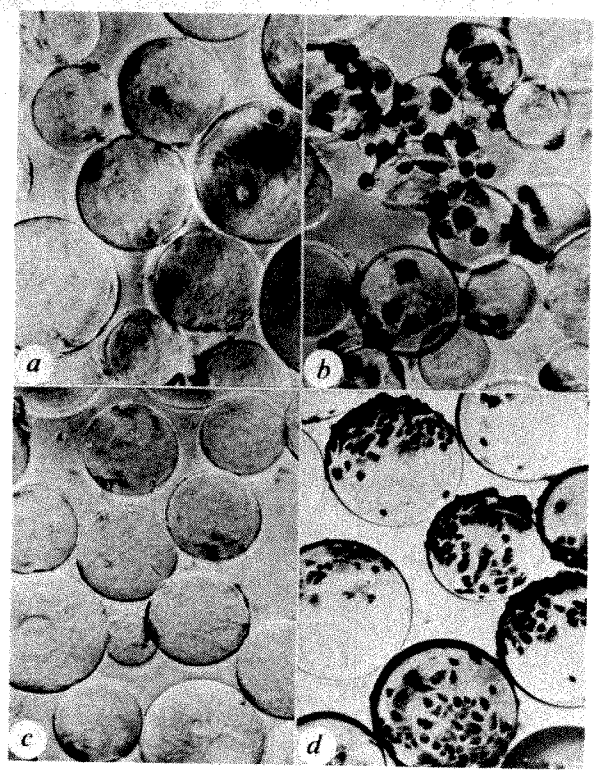


Fig. 3 Attachment of NRK cells to CH-Sepharose beads carrying tetrapeptides or fibronectin. Peptides TGRG (*a*), RGDS (*b*), GDSP (*c*) and fibronectin (*d*) were coupled to CH-Sepharose 4B (Pharmacia) by treatment of the Sepharose beads with 0.14 M 1-ethyl-3(3-dimethylaminopropyl)carbodiimide-HCl (Sigma) at *p*H 5–6 for 18 h at room temperature in the presence of a twofold molar excess of peptide relative to the available reactive groups on the beads. Coupling was quantitated by amino acid analysis and by monitoring the change in UV absorption. Each ml of Sepharose beads carried ~ 3–4 mg peptide or 10 mg of fibronectin. Attachment was assayed by incubating NRK cells with a monolayer of beads for 1 h at 37°C. The beads were then allowed to sediment (at 1 *g*) through 3 ml of 3% paraformaldehyde in phosphate-buffered saline to separate unattached cells and fix those cells which had attached. The unattached cells were decanted and the beads were stained with toluidine blue and observed under the microscope. *a*, *b*, and *c* ×50; *d* ×40.

Table 1 Proteins having a sequence similar to the cell attachment-promoting sequence of fibronectin

Proteins	Sequence	Ref.	Cell attachment
Fibronectin	A **V T G R G D S P** A S S K	3	Active
Fibrinogen α-chain	T **S Y N R G D S** T F E S K	4	Active
λ Receptor on *E. coli*	G S F G R G D S D E W T F	5	NT
Sindbis coat protein	G V G G R G D S G R P I M	6	NT
α Lytic protease	A C M G R G D S G G S W I	33	NT
Testis-specific basic protein	K S R K R G D S A D R N Y	34	NT

A computer search through published protein sequences was conducted by The National Biomedical Research Foundation. The proteins having the sequence Arg-Gly-Asp-Ser are listed and the sequences flanking the tetrapeptide are shown. Residues identical to those found in the fibronectin sequence are boxed; chemically similar residues are enclosed in broken boxes; and sequences which were tested as synthetic peptides for cell attachment activity are shown in bold type. A synthetic nonapeptide was prepared from the fibrinogen sequence and was found to be active (see Fig. 5). The other proteins have not been tested (NT; see text for discussion of these proteins).

in some way weaken the activity of this site so that it is not recognized by fibroblastic cells in the intact molecule. The Arg-Gly-Asp-Ser sequence in fibrinogen is flanked on both the COOH-terminal and NH_2-terminal sides by sequences which are more hydrophilic than the hydrophobic regions present on either side of this sequence in fibronectin. On the other hand, platelets interact with fibrinogen[19,20] at more than one site within the molecule[21], and they also interact with fibronectin[22]. Future work should show whether the Arg-Gly-Asp-Ser sequence of fibrin is recognized at any stage of haemostasis by platelets or other types of cells, or whether this sequence might become uncovered on enzymatic dissolution of a blood clot. Future work should also show whether this sequence mediates the interaction of any of the other proteins listed in Table 1.

Implications

Our data show that the minimum signal necessary for the binding of cells to fibronectin resides in the short amino acid sequence Arg-Gly-Asp-Ser and that each of the four amino acids contributes to the binding. That this sequence is common to a few other proteins which may interact with cells implies that cells may use a single mechanism (receptor?) for interacting with several proteins. Although no structural basis for the surprising activity of such a small peptide is readily apparent, some of its properties are suggestive. As already mentioned, the tetrapeptide is found in a segment of fibronectin that would be very likely to form a hydrophilic loop at the surface of the molecule[8,9] and would thus be available to interact with cells. As in our hands polycations are relatively ineffective compared with the synthetic peptides in promoting the attachment of fibroblasts and even less effective in promoting cell spreading (M.D.P. and E.R., unpublished results), it is clear that binding is not due solely to the arginine residue. In any case, the other three residues of the tetrapeptide are clearly important because substitution of the glycine residue with valine eliminates cell attachment activity, and omission or substituton of the aspartate alters the activity of the peptide drastically (Fig. 4). Furthermore, we have tested a synthetic peptide taken from a sequence in collagen[23], Lys-Gly-Glu-Ser-Gly-Ser-Pro, and found no cell attachment promoting activity (data not shown), indicating that a mere conservation of the charge relationships (Lys-Gly-Glu . . . versus Arg-Gly-Asp . . .) does not maintain the activity.

We have also tested the activity of other peptides that resemble the Arg-Gly-Asp-Ser sequence and are present in collagens[23]. One such sequence, Arg-Gly-Asp-Thr-Gly-Ala-Thr-Gly-Arg, (taken from type I collagen) promotes cell attachment almost as well as the fibronectin peptides; two other sequences (taken from collagens of different genetic types[23]), Gln-Gly-Ile-Arg-Gly-Asp-Lys-Gly-Glu-Pro and Gly-Ser-Arg-Gly-Asp-Hyp-Gly-Thr-Hyp, when tested as synthetic peptides had no cell attachment-promoting activity at all (unpublished data). These results indicate that the serine residue is not essential but only conservative substitutions of this residue are compatible with activity. The sequences flanking the Arg-Gly-Asp-Ser sequence also affect activity as illustrated by the fact that the Gly-Arg-Gly-Asp-Ser-Pro peptide can inhibit cell attachment to fibronectin better than the Arg-Gly-Asp-Ser peptide.

The data we have obtained by selective substitution within the recognition sequence reveal some interesting possibilities about modification of the fibronectin activity in cells and tissues. Based on these results, one can infer that a single point mutation in the fibronectin gene (for example, one that would change glycine to valine; Fig. 2) could destroy the activity of the recognition sequence. This raises the possibility that there exist fibronectins which are ineffective cell attachment promoters. Moreover, the cell attachment sequence may be subject to modification by phosphorylation. Fibronectin has been shown to carry covalently bound phosphate[24–26]. A partially phosphorylated serine has been found elsewhere in the fibronectin molecule within a region not showing internal homology with the cell attachment domain[26]. However, the phosphorylated serine is homologous

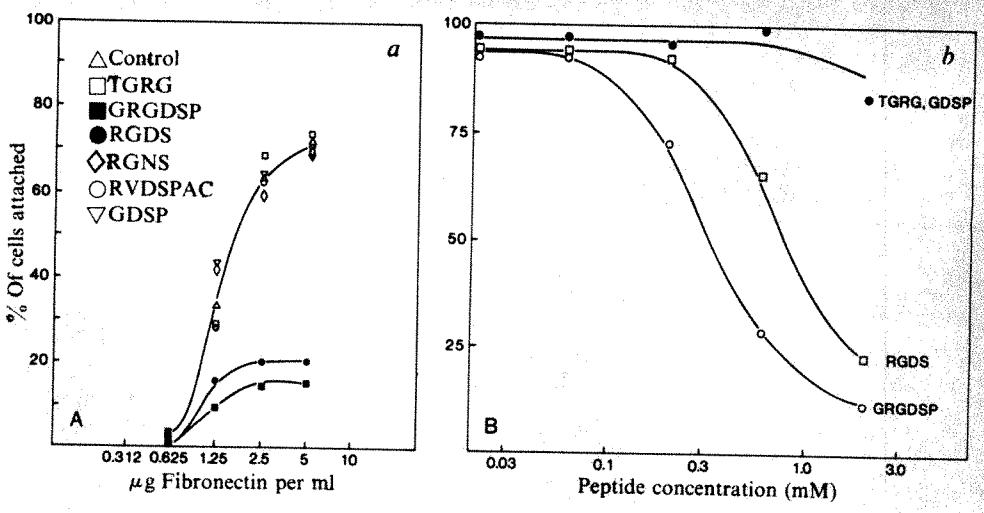


Fig. 4 Inhibition of attachment of NRK cells to immobilized fibronectin by synthetic fibronectin peptides. *a*, Microtitre wells were coated with decreasing concentrations of human plasma fibronectin, then non-bound fibronectin was washed away, and the attachment of NRK cells (10^4 cells per well) was assessed after incubation at 37 °C for 45 min in the presence of each of the peptides shown, each at a concentration of 1 mM. *b*, The peptides also were titrated in wells coated with 5 $\mu g\,ml^{-1}$ fibronectin. NRK cells were again incubated at 37 °C for 45 min, and attached cells were fixed, stained and counted. Incubation media for these experiments was Dulbecco's modified Eagle's medium (Flow) supplemented with glutamine–penicillin–streptomycin at 2 mM, 100 U and 100 $\mu g\,ml^{-1}$, respectively (Irvine Scientific) and bovine serum albumin 2 mg ml^{-1} (Sigma).

to the serine in the cell attachment site (compare Arg-Glu-Asp-Ser-PO_3 with Arg-Gly-Asp-Ser). Extrapolating from the data presented here, one would expect that the Arg-Glu-Asp-Ser sequence would not show cell attachment activity (compare Arg-Glu-Asp-Ser with Arg-Val-Asp-Ser). This agrees with our finding only one cell attachment site in each fibronectin polypeptide[1]. Whether the serine in the Arg-Gly-Asp-Ser sequence would be a suitable substrate for the same or a different protein kinase is unknown. In view of recent reports indicating the existence of cell surface protein kinase activity[27,28] and the finding of increased phosphorylation of fibronectin from malignantly transformed cells[29], this warrants further investigation. Phosphorylation could provide a means whereby cells might regulate their attachment and, where uncontrolled, could contribute to malignancy.

These results also raise the intriguing question of whether some prokaryotes and viruses use the fibronectin recognition sequence. The λ phage receptors on the surface of *E. coli* and Sindbis virus coat protein share a five-amino acid sequence with the cell attachment region of fibronectin (Table 1). It may be that some microorganisms use this sequence to interact with membranes of eukaryotic cells, and that some bacteriophages imitate the eukaryotic cell to gain entrance into *E. coli*.

The possibilities for future study discussed above are, at present, speculative. These possibilities, however, can be tested by using the peptides which inhibit cell attachment to fibronectin and, therefore, are also likely to interfere with other interactions which use this same recognition sequence. The peptides also provide us with the ability to specifically inhibit the cell attachment function of fibronectin, making it possible to study this function independent of the other functions of the protein in a variety of systems such as differentiation, cell motility, and in models of tumorigenesis and metastasis, all of which appear to be influenced by fibronectin[30–32].

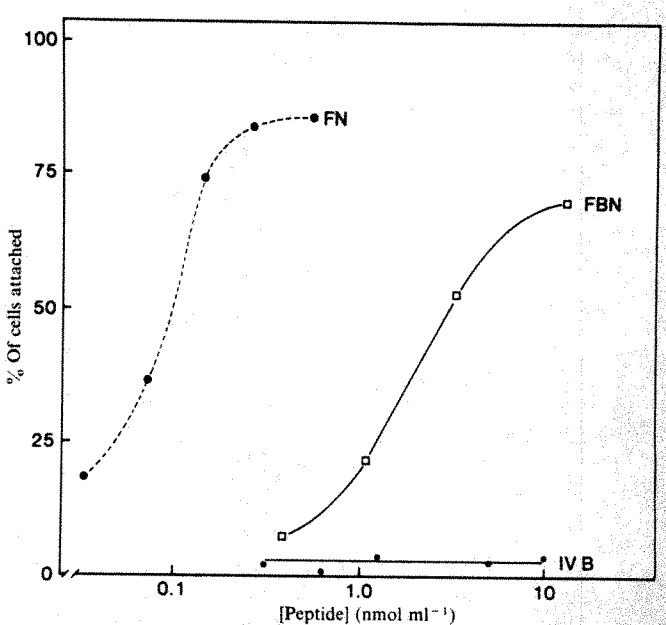


Fig. 5 Attachment of NRK cells to an immobilized synthetic peptide based on a sequence in fibrinogen. A synthetic fibrinogen peptide (FBN; see Table 1) was used to coat microtitre wells and was assayed for cell attachment as described in Fig. 2. Coating concentration of the peptide is indicated on the abscissa. The activities of fibronectin (FN) and peptide IVB are included for comparison.

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The Sunyaev–Zeldovich effect towards three clusters of galaxies

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An unequivocal detection of the Sunyaev–Zeldovich effect[1], the cooling of the brightness temperature of the microwave background radiation towards the atmospheres of clusters of galaxies, has profound cosmological significance[2–6], but has hitherto proved elusive. We present here measurements of the effect towards three rich clusters of galaxies made with the 40-m telescope of the Owens Valley Radio Observatory at 20.3 GHz. These results are three times more sensitive than other results previously reported[7–9], are free from systematic errors at the 100 μK level, and provide very strong evidence for the reality of the Sunyaev–Zeldovich effect.

The consensus of observational data supports the presence of a Sunyaev–Zeldovich effect towards only three clusters of galaxies—0016+16, Abell 665 and Abell 2218—although the support is by no means unqualified[7–9]. To provide further evidence for the reality or otherwise of the effect, we have worked for several years with the 40-m telescope of the Owens Valley Radio Observatory of the California Institute of Technology. Our results to 1983 January are reported elsewhere[10], but here we present the results of a successful observing run at 20.3 GHz made during the period 21 December 1983–3 January 1984.

For these observations, the 40-m telescope was equipped with a tunable K-band maser receiver with bandwidth ~400 MHz which provided an overall system noise temperature ~40 K. At our observing frequency the 40-m telescope has a beam efficiency of about 0.48, and a half-power beamwidth of 107 arc s. Observations were made using a symmetrical beam-switching scheme[10] with a beam-separation of 7.15 arc min. The weather was mostly good during our allocated observing period, and 237 h of useful data were obtained for the three clusters of galaxies 0016+16, Abell 665 and Abell 2218, and for nine reference regions of 'blank' sky located adjacent to the clusters or distant from them. These reference regions provide strong controls on the presence of systematic errors, which have severely handicapped earlier observations[11]. The data obtained from these observations were reduced using a modified form of the analysis of the variance[12]: tests with partial data-sets analysed in different ways gave consistent results. A test for parallactic-angle dependent systematic errors was also made[10], but none was found.

The results of these observations are given in Table 1, which contains the results for all the clusters and reference regions observed. The N and S positions associated with the clusters refer to points 7 arc min north and south of the cluster centres (the C positions, whose coordinates are given in ref. 10) respectively. The 0016+16 S position is contaminated by the presence of a nearby radio source whose contribution to the brightness temperature at this location is calculated (from lower-frequency measurements) to be 510±120 μK (refs 9, 13 and R. Laing, personal communication), and the brightness temperature measurement at this point has been corrected for this contribution. No corrections for the presence of radio sources have been applied to any of the other results in Table 1.

Because these observations were made using symmetrical beam-switching in azimuth, the ΔT_{RJ} brightness temperature measurements given in Table 1 represent the differences between the brightness temperatures towards the nominal points observed and the averages of points located on 'reference arcs' one beam-separation away, convolved with the primary beam-pattern of the telescope. For each cluster centre, the ΔT_{RJ} value therefore represents a measurement of a constant signal of ~0.45 of the central microwave decrement, ΔT_0 (using X-ray core sizes, θ_{cx}, of 0.50, 0.92 and 0.97 arc min for 0016+16, Abell 665 and Abell 2218 respectively, and a model

$$\Delta T_{RJ}(\theta) = \Delta T_0(1+\theta^2/\theta_{cx}^2)^{-\frac{1}{4}}$$

for the angular distribution of the decrement[10]). For the N and S reference locations, the reference arcs are located asymmetrically with respect to the cluster centres, and hence the cluster gas distributions, and cross the cluster centres at parallactic angle 90°. Averaged over the observational parallactic angle coverage, this implies that the N and S reference positions in these three clusters should see signals of only a few per cent of ΔT_0 appearing as small decrements or enhancements in the brightness temperature of the background radiation.

The differences between the values of ΔT_{RJ} measured at the cluster centres and at the N and S reference positions should, therefore, represent between 0.42 and 0.48 ΔT_0, and the significant depression of the C-position values of ΔT_{RJ} below those measured N and S of the cluster centres demonstrates the existence of significant Sunyaev–Zeldovich coolings towards each of the three clusters of galaxies.

Taking account of the parallactic-angle coverages of the observations, we can estimate for each cluster both the central microwave background decrement, ΔT_0, and any residual (constant) systematic offset signal, ΔT_s. These are given in Table 2. The values of ΔT_s are consistent with our limit of 100 μK deduced from the blank-sky results, but differ from zero at the 10% significance level even when correlations between ΔT_s and ΔT_0 in the fit are taken into account. For comparison Table 2 also gives the values of ΔT_0 deduced on the assumption that $\Delta T_s = 0$. Some small ΔT_s might be caused by pointing uncertainties or by the use of an inadequate model for the cluster atmospheres. Independent evidence for ~100 μK offsets has been found in other sensitive observations of the microwave background radiation using the 40-m telescope (A. C. S. Readhead, personal communication).

The values of ΔT_0 given in Table 2 correspond to significant detections of the Sunyaev–Zeldovich effect in each of these three clusters. The consequences of the detection of decrements with $\Delta T_0 = -0.7$ to -1.4 mK have been discussed elsewhere[10]—we note in particular that the very significant (8σ) detection of a

Table 1 The results

Cluster	Position	Brightness temperature change, ΔT_{RJ} (μK)	χ^2	Degrees of freedom	Fraction of data rejected in analysis
0016+16	C	−644±79	53.8	44	0.00
	N	+103±93	11.8	8	0.11
	S	−100±146*	17.0	10	0.15
Abell 665	C	−335±48	46.6	45	0.00
	N	−55±63	17.9	13	0.00
	S	−131±57	12.9	11	0.02
Abell 2218	C	−338±48	73.0	57	0.00
	N	+9±58	23.0	15	0.00
	S	−115±69	14.2	10	0.00
Blank sky	1	+6±74	48.1	23	0.00
	3	+189±86	39.1	26	0.00
	5	−126±96	21.4	9	0.00

The coordinates of the cluster centres, and of the blank sky regions, are given in ref. 10.

* +410±84 μK before correction for the radio source.

Table 2 Central microwave background decrements and systematic offsets

Cluster	Systematic offset signal ($\Delta T_s/\mu$K)	Central microwave background decrement with $\Delta T_s \neq 0$ ($\Delta T_0/\mu$K)	Central microwave background decrement with $\Delta T_s = 0$ ($\Delta T_0/\mu$K)
0016+16	+79±82	−1,584±256	−1,402±172
Abell 665	−89±44	−503±136	−694±98
Abell 2218	−40±47	−507±143	−700±98

microwave background decrement towards 0016+16 may provide a useful cosmological test[2–6].

We conclude that there should be no further doubt of the reality of the Sunyaev–Zeldovich effect in these three clusters of galaxies.

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Alignment of distant radio sources

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A typical extragalactic radio source is a simple double or elongated structure with a well-defined orientation on the sky. In 1972, Willson[1] investigated the relative orientation of 74 extragalactic 3C radio sources mapped at the Cambridge 1-mile telescope, and noted that extended extragalactic radio sources tend to be parallel if they are separated by less than 10° on the sky. Since 1972, many more radio sources have been mapped with resolutions 10–20 times greater than that of the observations available to Willson. I have therefore reinvestigated this problem in two separate and largely independent samples of some 300 sources each, and report here that the alignment is present to a high significance level, provided that only the more distant sources are considered. The effect could be due to gravitational imaging if most of the matter in a closed universe is in thin filaments.

In brief, the technique used by Willson is as follows. For a pair of radio sources one first determines the angular distance, D, measured along the great circle connecting the sources. If the axes of the radio sources make angles α and β with the connecting great circle, then the angle between the radio sources is defined as $X = \alpha - \beta$ reduced to the interval $0, \pi/2$. If $X = 0$, the sources are parallel; if $X = \pi/2$ they are perpendicular. Given N sources with measured position angles, one may calculate $\bar{X}(D_1, D_2)$ which is the mean value of X for all n_p pairs with separations between D_1 and D_2. If there exists no correlation of position angles, the distribution of X is uniform between 0 and $\pi/2$, and $\bar{X}(D_1, D_2) = 45° \pm 45°/\sqrt{3n_p}$.

Willson found that for all pairs with $D > 10°$ in his sample, $42° < \bar{X}(D) < 50°$. But for close pairs, $\bar{X}(D < 10°) \approx 36°$, which, given his relatively small number of close pairs was about two standard deviations below the expected mean of 45°. The probability of a 2σ fluctuation in his 11 separation intervals is about 10% which is, roughly, the significance of the effect.

The first of the samples studied here consists of published maps of identified extragalactic radio sources. The sample contains most of the 3C objects listed in the compilation of Laing *et al.*[2] with the position angles taken from the references therein, primarily from the Cambridge 5-km surveys[3–7]. The sample also contains objects from a published quasar survey[8] (mostly 4C objects) including more than 100 quasars observed at the Very Large Array (VLA) at resolutions of 1–2 arc s as well as a number of additional quasars compiled from earlier surveys[9–11]. Source position angles were measured directly from the maps in most cases, and strongly distorted sources with ambiguous position angles were excluded. Following Willson an additional selection criterion is applied to objects mapped with one-dimensional arrays. A source is removed from the sample if its angular size is smaller than two north–south half-power beam-widths because inclusion of such sources would introduce an east–west bias in the position angle distribution.

With these conditions the total sample consists of 320 sources, of which 177 are identified with quasars and 143 with radio galaxies. Carrying out the analysis described above, I find only a very marginal effect. For separations smaller than 5°, $\bar{X}(D < 5°) = 42.76°$, which is a fluctuation of 1.33σ below the expected value of 45°. But considering only the sources with redshifts greater than 0.4 (191 sources) the results are quite different, as seen in Fig. 1 which is $\bar{X}(D)$ binned in 5° intervals. Here we find that $\bar{X}(D < 5) = 36.37°$, which is a deviation of 3.4σ. The distribution of X for all sources with separations smaller than 5° is shown in Fig. 2. This distribution obviously deviates significantly from uniform with more sources tending to be parallel. From the Kolmogorov–Smirnov test[12] the probability that the

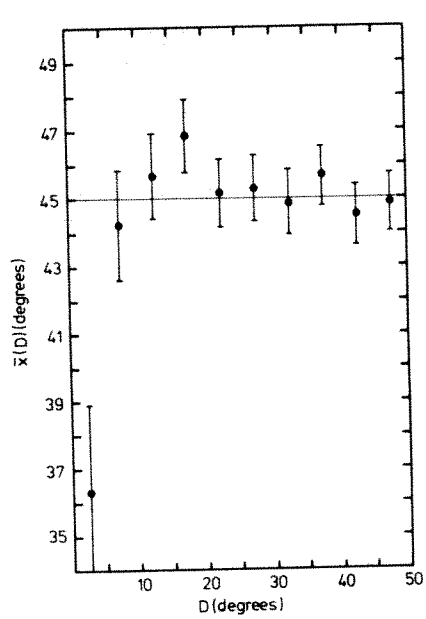


Fig. 1 The mean angle between radio sources in degrees as a function of angular separation in degrees for all sources in the 3C–4C sample with $z > 0.4$. The average is taken over 5° intervals and the error bars indicate the ± standard deviation for a uniform distribution ($45°/\sqrt{3n_p}$ where n_p is the number of pairs in a bin).

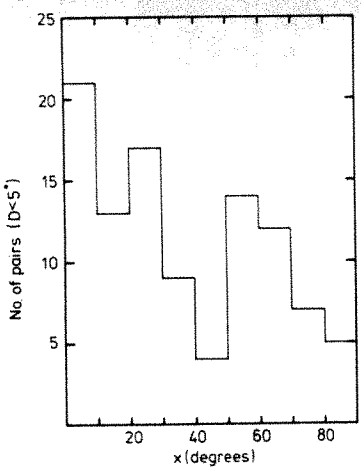


Fig. 2 The distribution of X, the angle between sources, for 102 pairs of sources in the 3C–4C sample closer than 5° on the sky and with $z > 0.4$.

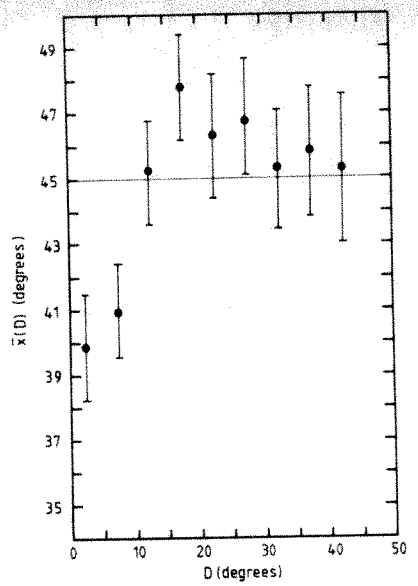


Fig. 3 The same as Fig. 1, but for all sources in the B3 sample smaller than 42 arc s.

observed distribution of angles between closely separated sources is drawn from a uniform random distribution is 0.003. This has been verified by Monte Carlo simulations in which source positions are unchanged but position angles are scrambled. The probability of finding such a large deviation over the entire range of separations (0°–50°) is roughly 10 times larger or about 3%.

As all sources in this 3C–4C sample have measured or estimated redshifts, it is possible to determine whether or not sources in nearly parallel pairs at close separations are at the same distance. There are 34 pairs in which the sources are closer than 5° and are parallel to within 20° (that is, $D < 5°$, $X < 20°$). The distribution of Δz, the redshift difference between the two members of these pairs, does not differ significantly from that of 34 pairs drawn randomly from the sample. For only four of these pairs is $\Delta z < 0.1$. This implies that the nearly parallel sources with $D < 5°$ which produce the effect are in general not physically close together.

A second largely independent and more homogeneous sample is provided by recent VLA observations of more than 1,000 sources selected from the third Bologna survey (B3) (R. Perley, B. G. Clark, A. H. Bridle and G. Greuff, in preparation). All mapped sources lie within a narrow declination range (38°–48°), and the observations were done in a mode which assured a circular beam with a half-power width of 14 arc s. Therefore there should be no systematic false effects resulting from an elongated beam. Of the mapped sources roughly one-third have structure on a scale larger than the beam. Position angles were measured for 297 sources with simple double or linear structure. Most of the sources in this sample are unidentified so redshift restrictions cannot be made.

In the complete sample there is no tendency for alignment. For separations less than 5°, we find $\bar{X}(D < 5°) = 44.66°$, which is within 1 s.d. of the expected value. Although a redshift restriction is not possible for the B3 sample, an angular size restriction is in some sense equivalent. By eliminating large sources from the sample, one eliminates nearer sources although the sample is still contaminated by small nearby sources. If all sources larger than 42 arc s (three beam widths) are removed, the results are shown in Fig. 3 which again is $\bar{X}(D)$ averaged in 5° bins. Although there are only 94 sources smaller than 42 arc s, there are still many close pairs because of the very restricted sky coverage of the survey. Here again we see a very significant tendency toward parallelism at small separations: $\bar{X}(D < 5°) = 39.84°$, or a 3.2σ deviation. Moreover, in this sample, the effect of alignment seems to extend to 10° with $\bar{X}(D < 10°) = 40.48°$, a 4.3σ deviation. The distribution of X for pairs separated by less than 10° deviates from uniform at the level of 7×10^{-5} (data not shown). The probability for finding such a large deviation at any separation is 0.1%.

Any subtle instrumental effect which might produce a spurious alignment (such as, for example, some aspect of the telescope side-lobe pattern giving greater sensitivity for extended structure in certain orientations) would be expected to show up as a non-randomness in the distribution of position angles. In both restricted samples taken separately the distributions of position angles do deviate somewhat from uniform at significance levels of about 10%. To test whether or not non-uniformity of the position angle distributions of this order could result in apparent alignment of closely separated sources, I have generated random skys in which the overall distribution of position angles is exactly the same as that observed; that is, the position angles are not scrambled but redistributed among sources. The frequency of chance alignments as significant as those observed is less than 1/1,000 in both samples implying that the effect does not result from non-uniformity in the global position angle distribution. It is more likely that the small non-randomness seen in the position angle distribution is an effect of the presence of several groups of closely separated well-aligned sources in samples of 100 to 200 objects.

The fact that the very pairs of objects which give the effect its high significance are widely separated in space (at least in the 3C–4C sample) implies that the effect arises along the line-of-sight. A possible explanation is gravitational imaging by superclusters[13]. Superclusters seem to be generally linear structures 10 to 100 Mpc in length[14]. The amplification produced by linear lenses is one-dimensional; the image of a background source is stretched in a direction perpendicular to the supercluster axis. One-dimensional amplification of a group of randomly oriented background sources can produce significant net alignment if most of the matter in an $\Omega_0 = 1$ universe lies in thin (<1 Mpc) long (~50 Mpc) filaments[13].

In view of the possible cosmological implications it is imperative that the effect should be confirmed. Remapping of the B3 radio sources at a resolution of 1–2 arc s is underway at the VLA and should definitely establish (or rule out) the reality of the apparent alignment. An additional experiment would consist of mapping all the radio sources down to some low flux limit in a small region of the sky (a $3° \times 3°$ field for example). If the effect is real, then the distribution of source position angles should deviate significantly from uniform.

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The case for antiparticles in the extragalactic cosmic radiation

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The presence of an excess of low-energy antiprotons in the primary cosmic radiation[1–3] has given rise to several possible explanations[4], some of which involve exotic processes such as mini-black holes and extragalactic antiparticles. Here we consider the latter possibility[5] and show that there are interesting implications for the cosmic radiation at higher energies. Indeed, it may be possible to account for a previously puzzling feature of the cosmic ray spectrum—a 'bump' in the range 10^{14}–10^{15} eV—by hypothesizing a primary extragalactic origin for the bulk of the observed cosmic ray antiprotons, although such an explanation is not unique. In this model most of the cosmic rays above 10^{15} eV are extragalactic. We prescribe a method of testing this hypothesis experimentally.

One of the most fundamental questions in cosmology is the question of the existence of antimatter in significant quantities in the Universe. Does antimatter have an equal role with matter in the makeup of the galaxies? This question has now become one of fundamental importance to physics also. In the contemporary paradigm of grand unified gauge theories it is related to the question of the nature of charge parity violation at high energies[6]. Early attempts at constructing a baryon symmetric cosmology[7,8] suffered from the difficulty of providing an effective mechanism for separating matter and antimatter on ultimately large enough scales to be consistent with astronomical observations. However, recent theoretical work based on the concepts of grand unified theories (GUTs) has led to the development of a plausible baryon–antibaryon domain theory in which matter and antimatter are created in separate regions of survivable size to begin with (refs 9–11 and A. Mohanty, in preparation). Various observational aspects of this theory have been discussed previously[12,13] and the subject of baryon symmetric cosmology has been reviewed recently[14,15]. It should, of course, be noted that regardless of the ultimate fate of our present theoretical ideas either in favour of or against the reality of baryon symmetry on an overall universal scale, the experimental search for primary cosmologically significant quantities of antimatter is a task of deep significance. Further, both the recent theoretical work and the reports by various groups of surprisingly large fluxes of antiprotons in the cosmic radiation give immediacy to this search.

The present status of cosmic ray antiproton ($\bar{p}$) measurements and the attempts to understand them have been recently reviewed[4] and an exegesis of the primary extragalactic origin hypothesis has also been given recently[5]. Here we will discuss further implications of potential basic importance to cosmic ray research and we will propose an experimental search programme based on these considerations. We start with the hypothesis that the baryon symmetric domain cosmology leads to a flux of extragalactic cosmic rays consisting of roughly equal amounts of protons and antiprotons with the sources of these cosmic rays being primarily active galaxies[16,17] and with helium and antihelium nuclei being suppressed by destruction processes in these sources[5]. (We will also assume that helium and antihelium nuclei exist in the extragalactic cosmic rays at a lower level, supplied by leakage from normal matter galaxies and normal antimatter galaxies.) We further assume that the galactic wind is too weak to keep out the extragalactic cosmic radiation. Observations favour the interpretation that the galactic wind is in reality a 'breeze'[18].

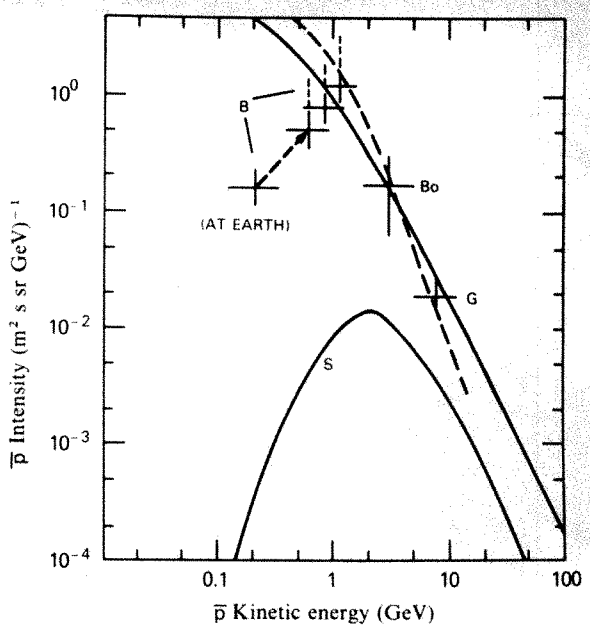


Fig. 1 Observations and theoretical $\bar{p}$ spectra. The data points are: B, from Buffington *et al.*[3]; Bo, from Bogomolov *et al.*[2]; and G, from Golden *et al.*[1]. The higher points marked B take account of solar modulation effects[24]. The solid curve (S) is for the secondary $\bar{p}$ production model with mean propagation length 5 g cm^{-2}, and the dashed line corresponds to the shape of the $\bar{p}$ spectrum calculated for a primordial black hole model[24]. The higher solid line corresponds to our spectrum for the extragalactic primary hypothesis as discussed in the text.

Let us first consider the energy spectrum of the cosmic radiation. The measured spectrum of cosmic radiation can be represented by a power law in energy of the form $KE^{-\Gamma}$ with the spectral index $\Gamma \simeq 2.75$ for several decades above the 10 GeV energy level. It seems likely that this radiation is produced primarily in galactic sources[19,20]. Furthermore, the source spectrum of this radiation is expected to have a lower spectral index, Γ_s, than that observed at the Earth which has been steepened by energy-dependent propagation effects. A value for Γ_s of ~2.0 to 2.2 appears to be likely for two reasons. (1) Measurements of the ratio of secondary to primary nuclei in the cosmic radiation suggest that the mean lifetime in the Galaxy due to trapping by the tangled galactic magnetic fields falls with energy as $E^{-\delta}$ where the most recently derived value[21] of δ is about 0.7. (2) The theoretical shock acceleration models for cosmic ray production that are presently favoured[22] generally yield production spectra with Γ_s close to 2.

If we assume that there exists a general acceleration mechanism for generating cosmic rays which acts in both galactic and extragalactic sources to give a universal source spectrum with $\Gamma_s \simeq 2$, as is now thought to be the case with shock acceleration, the extragalactic cosmic ray component should reflect this source

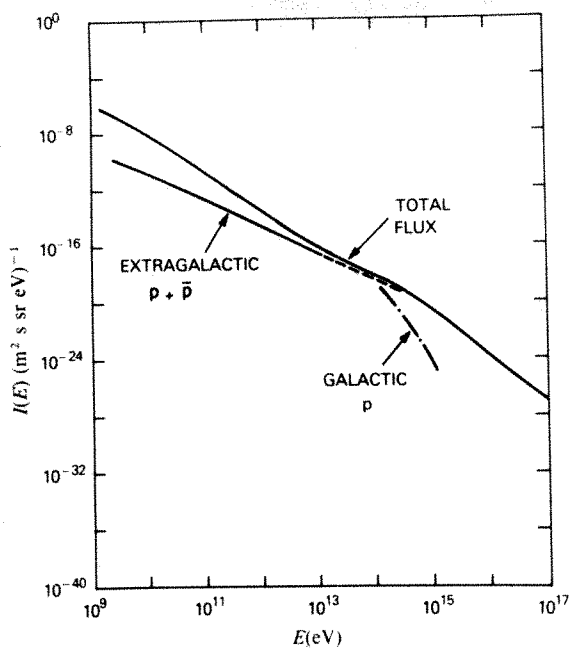


Fig. 2 The effect of extragalactic primary protons and antiprotons on the total cosmic ray spectrum according to the model discussed in the text. It can be seen that this model may account for the putative flattening in the observed cosmic ray spectrum near 10^{14} eV. $I(E)$ is the particle intensity, in the units shown.

spectrum. Thus, with the antiprotons assumed to be both primary and extragalactic and the bulk of the protons assumed to be galactic, the expected ratio of antiprotons to protons should increase with energy as E^{δ}. Taking $\delta \simeq 0.7$, antiprotons could make up approximately 1% of the cosmic ray flux at an energy of $\simeq 500$ GeV and even $\sim 50\%$ at higher energies; this has important observational implications which we will discuss later. Figure 1 shows the present data on antiproton intensities and a theoretical extrapolation corresponding to a primary extragalactic antiproton flux with $\Gamma = 2.0$. For these considerations, we assume that secondary production of antiprotons is unimportant (see, for example, the estimate given in Fig. 1).

Figure 2 shows the effect of extrapolating the extragalactic intensity of both protons and antiprotons (this introduces a factor of two) with a spectral index of 2 to higher energies and superposing it on the galactic cosmic ray spectrum with index $\Gamma \simeq 2.75$. Note that such an extrapolation implies that the extragalactic and galactic cosmic ray fluxes become comparable at an energy of $\sim 10^5$ GeV and that extragalactic particles predominate above this level. It is interesting to note that the resultant flattening in the spectrum occurs at this particular energy (10^5 GeV) where there have been claims[23] of a flattening in the cosmic ray spectrum as inferred from measurements of extensive air showers. It should, of course, be noted that a steepening in the spectra of both the galactic and extragalactic components would be required by the observations for energies above 10^6 GeV but this is not difficult to explain.

As our model indicates that the antiproton-to-proton ratio should increase with energy, measurements of the sign of the charges of cosmic rays at the highest practical energy and the determination of the spectra of the various charged components of the cosmic radiation up to that energy will provide a test of our hypothesis. Such a test requires that the experiment be placed above the atmosphere so that the incoming cosmic ray nuclei can be measured directly. Furthermore, the sign of the charged particles (and their magnitude) may be measured by using a superconducting magnet. Such an experiment, with an attainable energy of ~500–1,000 GeV, could be flown aboard a space shuttle (T. Bowen *et al.*, unpublished NASA research proposal). In addition, an emulsion stack experiment could be flown on a high-altitude balloon or on the space shuttle to look for antihelium nuclei, even at the reduced level implied by our hypothesis. A polar or near-polar orbit would be desirable to avoid the geomagnetic cutoff. In view of the almost impossible odds of creating a secondary ^{4}He antinucleus, the unambiguous detection of even one such particle would provide irrefutable evidence of primary cosmic ray antimatter. (It should be noted also that the production of 100–300 MeV antiprotons is extremely difficult because of well known kinematical factors[5]. This feat has proved elusive even to accelerator physicists who have deliberately set out in the laboratory to make such particles (C. Rubbia, personal communication). Thus, the observed low-energy antiprotons in the cosmic radiation are also quite difficult to explain as secondaries from cosmic-ray interactions.)

The experimental programme proposed above should add to our basic understanding of $\bar{p}$ origin. If the $\bar{p}/p$ ratio is observed to increase as $\sim E^{0.7}$, our hypothesis of extragalactic antiprotons from antimatter galaxies will receive strong support. The observation of antihelium nuclei would be conclusive. The extent to which non-observation of antihelium disproves our hypothesis is unclear, but if $\bar{\alpha}/\alpha \ll 10^{-5}$ (the value expected very approximately on the basis of $\bar{\alpha}$s leaking from 'normal' antimatter galaxies), the difficulty would be severe.

Non-observation of antihelium (at the level given above), together with an increase in the $\bar{p}/p$ ratio with energy at a slower rate than E^{δ} (for example, $\propto E^{0.25}$ between 10 and 100 GeV), would be consistent with the closed galaxy model (or source trapping), although there are severe theoretical problems[5,24] with such a model.

Finally, the observation of a $\bar{p}/p$ ratio falling rapidly with increasing energy would support the black hole model of Kiraly *et al.*[24] (see Fig. 1).

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Neutrino tomography: Tevatron mapping versus the neutrino sky

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Volkova and Zatsepin[1] were the first to propose the use of a 'Tevatron' (a high-energy accelerator at 10^{12} eV or TeV energies) to beam neutrinos ν through the Earth to a mobile detector and obtain X rays of the planet's interior. The basic idea has been contingent for years on the opacity of the Earth to neutrinos at appropriate energies (10–10^3 TeV). This depends significantly on the neutrino–nucleon scattering cross-section, which in turn depends on the existence of an intermedi-

ate vector boson[2–4], the acceptance of what physicists refer to as the standard model[5–7] in high-energy physics, and finally the mass M_W of the intermediate W boson in that model. The announcement by CERN of the W boson mass[8,9] ($M_W \sim$ 80 GeV) permits us now to draw certain conclusions. Although we shall demonstrate that neutrino tomography at planetary densities is feasible, tomographic scanning schemes appear unrealistic in several respects. We shall address two sources of neutrinos for tomographic mapping of the densities, the Earth-based Tevatron and the neutrino sky.

Based on arguments from computerized tomography[10–13], group discussions[14] of future elementary particle facilities[15] (also in presentations of satellite tomography, Fig. 1) pointed out that 'X-raying' the Earth with neutrinos cannot provide a proper solution to its composition and density for the same reasons that single-projection X rays do not constitute an adequate reconstruction for noninvasive imaging in medicine. Geophysical densities follow from the mapping of the Radon or Fourier transform of certain neutrino projections, and not from the Volkova–Zatsepin scheme, as we shall demonstrate.

Tomographic mapping of the Earth is illustrated in Fig. 1, using deep underwater muon and neutrino detectors[16–18] (DUMAND). It measures a known distribution of ν radiation passing through an object, defining this as a projection χ, and taking the Fourier transform $F(u, v)$ of the projection over the two-dimensional Fourier space (u, v). The inverse transform determines the densities. It has been described in detail[12,13,19–21] and, in fact, is directly related to the very long baseline interferometry techniques used in radio astronomy[21–23]. Two source distributions are depicted: a parallel-beam projection generating a planar slice of data F_i through $F(u, v)$ (Fig. 1*a*, *b*), and a fan-beam projection in Fig. 1*c*, *d* generating a cylindrical slice $\tilde{F}_i$ of data on $F(u, v)$. Many multiple projections are required to map or 'fill in' the surface $F(u, v)$. in Fig. 1*b*.

Treated mathematically, the mapping problem was first addressed by Radon[24] who derived what has now become known as the Radon transform. Tomography[10,19,20,25] can be defined as reconstructive imaging by means of the inverse Radon transform technique, its relation to the Fourier transform having been extensively discussed[19,20,26–29]. Whenever the Radon or Fourier transform is inadequately determined, its inverse cannot be properly defined and the scheme is described as a mathematically ill-posed inverse transform problem[20].

The Volkova–Zatsepin Tevatron scheme, also proposed[30,31], is illustrated in Fig. 1*c*, which shows that an infinite number of fan-beam measurements using an infinite number of detectors or detector positions only maps one cylindrical slice $\tilde{F}_1$ of the Fourier transform for the Earth-fixed Tevatron A. We conclude from Fig. 1 that the geographically fixed Tevatron scheme of Volkova–Zatsepin, and its detector counterpart in Fig. 1*d*, constitute ill-posed inverse Radon transform problems, and therefore do not work[1,31] except as X-raying schemes, which have limited use in geophysics because they do not determine the densities. (The mobility referred to in ref. 31 is for geophysical prospecting, not for solution of the inverse Radon transform problem.)

The well-posed inverse Radon transform problem may be realized as follows. The source distribution must be known and measurable, and the neutrino source and detector must both rotate relative to the Earth, mapping an appropriate part of $F(u, v)$. The dashed circle in the u–v plane of Fig. 1*c*, *d* can be visualized as a fan-beam 'bubble' in momentum space located beneath the Tevatron A in real space. As A moves geographically, the bubble rotates about the origin of Fourier space and maps $F(u, v)$. Well-posed mapping schemes can be improvised from Fig. 1.

Having a well-posed scheme, the opacity of the Earth must be estimated. It can be determined from seismic models of the Earth's density distribution. Standard models have been described by Press[32] and Dziewonski and Anderson[33], supplemented by extensive work on the geochemical composition of the Earth by others[34–37]. We adopt the Dziewonski–Anderson preliminary reference earth model (PREM) here primarily because it states explicitly the radial density functions ρ. It is compatible with the Monte Carlo study of Press[32]. The results are given in Table 1 and illustrated in Fig. 2. Figure 3*a* will be used in the derivation.

The PREM density distributions define zones or concentric shells[33]. Cords of path length L at angle ψ from the nadir were chosen tangent to each shell at a depth $d = a(1 - \sin\psi)$, where $a = 6{,}371$ km is the radius of the Earth. By means of a simple coordinate transformation, line integrals were then performed along L to determine the total nucleon mass $M = M(\psi)$ in a 1 cm^2 cylindrical column at angle ψ through the PREM model:

$$M(\psi) = \int \rho(\psi, L)\, \mathrm{d}L \qquad (1)$$

(ρ in equation (1) is actually measured in ν-tomography by the inverse Radon transform technique already described.) The total mass was next divided by the mean mass per nucleon $\bar{m}_n$ to

Table 1 Neutrino tomography, from the PREM Earth model

PREM zone (density g cm^{-3})	ψ (deg)	n ($\times 10^{34}$)	L (10^4 km)	$N = n/L$ (10^{24} cm^{-3})	$\kappa = \sigma_{\nu N} N$ (10^{-10} cm^{-1}) 1 TeV	10″	100″	$\lambda = \kappa^{-1}$ (Earth radii) 1 TeV	10″	100″	% Attenuation $(1 - I/I_0) \times 100$ 1 TeV	10″	100″
Central core (13.09–12.70)	0.0	0.6600	1.2742	5.1796	0.33	2.3	9.8	48.1	6.7	1.6	4.1	25.7	71.5
Outer core (12.17–9.90)	11.05	0.5362	1.2506	4.2877	0.27	1.9	8.1	58.1	8.1	1.9	3.3	21.4	63.9
Lower mantle (5.57–4.38)	33.11	0.3770	1.0674	3.5511	0.22	1.6	6.7	70.2	9.8	2.3	2.4	15.7	51.3
Transition zone (3.99–3.98)	63.49	0.1774	0.5688	3.1183	0.20	1.4	5.9	79.9	11.2	2.6	1.1	7.7	28.6
Transition zone (3.98–3.72)	64.93	0.1634	0.5398	3.0273	0.19	1.36	5.8	82.3	11.5	2.7	1.0	7.1	26.7
Transition zone (3.54–3.44)	69.59	0.1084	0.4444	2.4385	0.15	1.1	4.6	102.2	14.3	3.4	0.7	4.8	18.6
Low velocity zone (2.69–3.38)	74.90	0.0694	0.3320	2.0900	0.13	0.9	4.0	119.2	16.7	4.0	0.4	3.1	12.4
Crust (2.90)	84.98	0.0180	0.1114	1.6191	0.10	0.73	3.1	153.9	21.5	5.1	0.1	0.8	3.4
Crust (2.60)	86.08	0.0128	0.0874	1.4669	0.09	0.66	2.8	169.9	23.8	5.6	0.08	0.6	2.4
Ocean (1.02)	88.25	0.0024	0.0391	0.6150	0.04	0.3	1.2	405.1	56.7	13.4	0.0	0.1	0.5

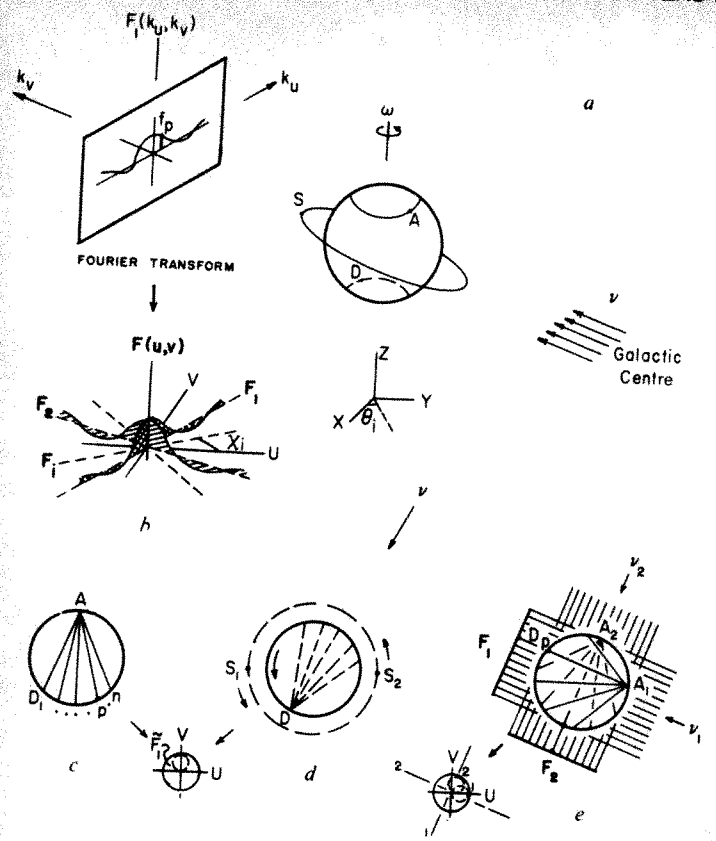


Fig. 1 Neutrino tomography represented as an idealized problem of mapping the Fourier transform $F(u, v)$ of projections of high-energy neutrinos ν through the Earth. *a*, With the Earth fixed ($\omega = 0$) in the inertial frame XYZ a ν-detector S orbiting in the galactic plane could generate one slice $F_i(u, v)$ of the transform in two-dimensional momentum space (k_u, k_v) of Fourier space (u, v). *b*, The actual Earth rotates in the inertial XYZ frame ($\omega \neq 0$) about the Z-axis shown, and for any Right Ascension θ_i, S would generate another slice F_i, gradually mapping $F(u, v)$. *c*, An Earth-fixed fan-beam scanning scheme is shown consisting of an accelerator A, beaming TeV neutrinos through the Earth to a mobile ν-detector D. *c* Is mathematically ill-posed; it can generate only one cylindrical slice $\tilde{F}_1$ of points on the surface $F(u, v)$, depicted by the dashed circle (a polar vector of magnitude $a \sin \psi$) in the u–v plane of *c*. *d*, Another ill-posed scheme using an Earth-fixed detector D which scans the Earth's interior while the Earth rotates about the celestial sphere. Schemes *c* and *d* pose acceptable imaging configurations only when A and D are moved. *e*, Part of a well-posed global ring of collaborative Tevatrons, or mobile positions. $\tilde{F}$ is constructed by knowing that $A_1D_p \rightarrow f_p$ is measured by the same fan or parallel beam. To visualize measuring the density of one point P* within the Earth, its contour of data on $F(u, v)$ is the smallest cylindrical slice $\tilde{F}^*$ (that is, a smaller dashed circle like that in *c*) osculating with P^* and the centre of the Earth. $\tilde{F}^*$ is measured by moving A on the surface and measuring values of $\tilde{F}$ in *c* where it intersects $\tilde{F}^*$ while $\tilde{F}$ rotates around the u–v origin.

derive the number of nucleons n in the column L. That is, $n = \bar{m}_n^{-1} M$ where $\bar{m}_n^{-1} = 6.029030 \times 10^{23}\ g^{-1}$ is approximately Avogadro's number N_A, and was derived from refs 38, 39 assuming an equal number of protons and neutrons, an average binding energy per mass number of 8.5 MeV and mass numbers of 24 for the mantle and 46 for the core[36,37].

Dividing the nucleon number n by the volume of the column which is $L\ cm^3$, we derive the nucleon density N averaged along the cord: $N = n/L\ cm^{-3}$. Given a neutrino–nucleon scattering cross-section $\sigma_{\nu N}$ (an area in cm^2), the neutrino's opacity κ_ν follows dimensionally as the product of $\sigma_{\nu N}$ and N:

$$\kappa_\nu = \sigma_{\nu N} N = \sigma_{\nu N}\, n/L \quad cm^{-1} \tag{2}$$

The neutrino's mean free path is simply the inverse of its opacity: $\lambda_\nu = \kappa_\nu^{-1}$ cm.

The issue of critical importance in equation (2) is the energy dependence of $\sigma_{\nu N}$. At energies below 1 TeV, experimental groups[38–41] have corroborated the assumption that $\sigma_{\nu N}$ increases linearly with neutrino energy E_ν,

$$\sigma_{\nu N} = 0.67 \times 10^{-38}\, E_\nu \quad cm^2 \tag{3}$$

where E_ν is in units of GeV $= 10^{-3}$ TeV. Equation (3) is known as the Bjorken scaling hypothesis[42,43]. It is shown graphically as the dashed straight line in Fig. 3*a*, and is predicted by the ordinary four-fermion theory of the weak interaction[44]. It is equivalent to the assumption that $M_W = \infty$, and was used in refs 1 and 31. However, a fundamental tenet of particle physics is that equation (3) cannot be valid at arbitrarily high energies, because it eventually violates what physicists refer to as the unitary of the scattering matrix[44,45]. It was shown[46] that the deep-inelastic cross-section levels off logarithmically at higher energies due to virtual W boson propagator effects (equation (6.4) of ref. 46),

$$\sigma_{\nu N} \sim \log E_\nu \tag{4}$$

instead of increasing linearly as in equation (3).

Theoretical studies of neutrino cross-sections have shown the Bjorken–Paschos effect in equation (4) to be prevalent at tomographic energies (10–10^4 TeV). Original concern was with the production of real W bosons[47–54] by elastic scattering from target nuclei[47–51] (coherent) or individual nucleons[48–51] (incoherent) at small values of the W boson mass ($M_W \sim 5$ GeV). As estimates for M_W increased ($M_W \sim 80$ GeV), the earlier results[49] were revised[52–54] for elastic W boson production while interest continued in the deep-inelastic case[41,52–55] where the target nucleon breaks up into hadronic debris X (for example, $\nu + N \rightarrow \mu + X$). It involved the production of virtual (not real) W bosons[41,51–54], and was shown[51–54] to be the dominant[52,53,55] effect at the energies considered here.

We adopt the values for $\sigma_{\nu N}$ as those stated by Halprin[53] using the quark–parton distributions of Buras and Gaemers[41,56] for $M_W = 80$ GeV as announced by CERN[8,9], with a Bjorken–Paschos logarithmic extension from equation (4). This is shown graphically in Fig. 3*a*, which is the basis for Figs 2 and 3. (Had M_W been much smaller, $\sigma_{\nu N}$ in Fig. 3*a* would level off as shown by the dotted curve at a value too low for ν-tomography to work for planetary densities.)

Tomography (Fig. 2) is concerned with the measurement of the transmitted intensity I of neutrinos as compared with the incident intensity I_0 (refs 10, 11). I_0 must be known. These are related to κ and λ by

$$I = I_0\, e^{-\kappa L} = I_0\, e^{-L/\lambda} \tag{5}$$

as a function of path length L through the Earth. The attenuation is $(1 - I/I_0)$ and is given in Fig. 2 as a percentage for 1, 10 and 100 TeV using the PREM values. The Earth is still virtually transparent at 1 TeV, tomography of the core is possible at 10 TeV and imaging out into the upper mantle is possible at 100 TeV, if we assume a minimum attenuation of 10% to account for measurement uncertainties. Surface tomography[31] below 100 TeV at PREM densities appears meaningless (except for very dense anomalies there) because the mean free path exceeds 5 Earth radii at that energy (Table 1).

Because tomography cannot be performed without a knowledge of the source distribution I_0, Tevatron tomography offers the distinct advantage that I_0 can be accurately measured and controlled. On the other hand, we have seen that the inverse Radon transform problem requires an unrealistic, global distribution of Tevatrons and DUMAND-type detectors. We now address the question posed by Fig. 1*d* of the existence of a tomographic sky, treating ν-tomography as a discipline of astronomy and requiring only the detectors. Neutrino tomography might be feasible as an analysis of the nadir data cuts, using I_0 determined from the zenith data cuts in neutrino astronomy.

Natural sources, predicted in the neutrino sky, are illustrated in Fig. 3, adapted from Margolis, Schramm and Silberberg[57,58]

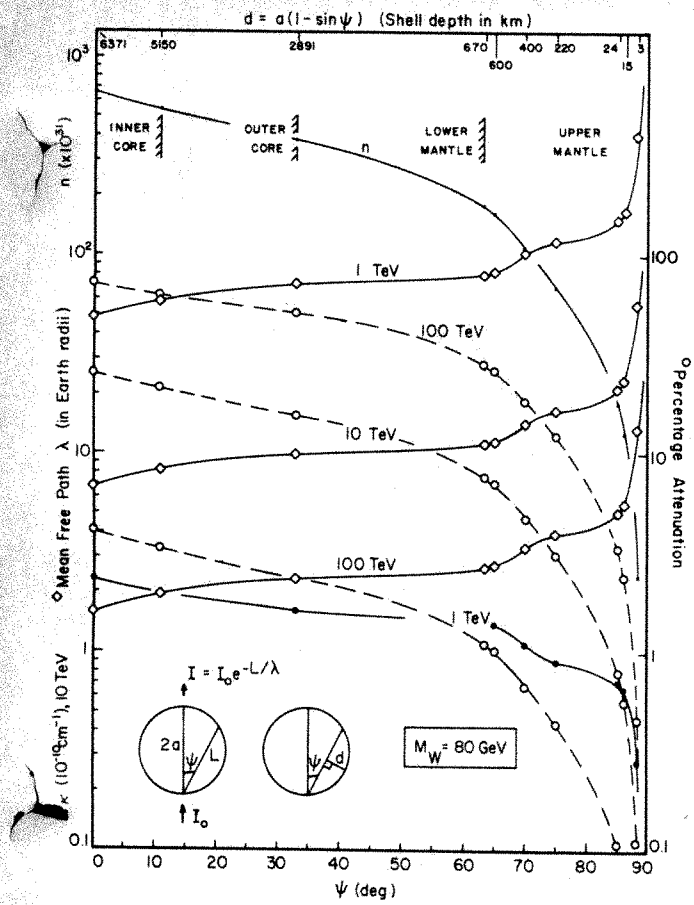


Fig. 2 Opacity of the Earth to neutrinos, derived from the PREM density model of ref. 33. The number of nucleons n in a cylindrical column 1 cm^2 in area along a cord of length L is plotted as a function of the angle ψ from the nadir. From n and L a neutrino opacity κ is derived as a function of neutrino cross-section (shown in Fig. 3a) and therefore energy. The mean free path of the neutrino $\lambda = \kappa^{-1}$ is shown by solid lines, and the corresponding percentage attenuation is given as the dashed lines at the same energies. Tomography 'works' when the attenuation exceeds some threshold value (assumed here as 10%, see ref. 18, p. 235) which accounts for measurement uncertainties. Neutrino oscillations[16–18] have been neglected. (An actual beam of neutrinos from an accelerator diverges, forming a conical frustrum whose volume is approximately $AL/3$, where A is the area of the ν-beam as it leaves the Earth; the number of nucleons n' in the beam's volume is $n' = An/3$. Although n' is $10^{10}/3$ times n for $L = 2a$ and $A = 1\ km^2$ using a π-meson source in ref. 1, mean nucleon density is unchanged. Tomographic resolution is sacrificed as n' increases for different ν-sources.)

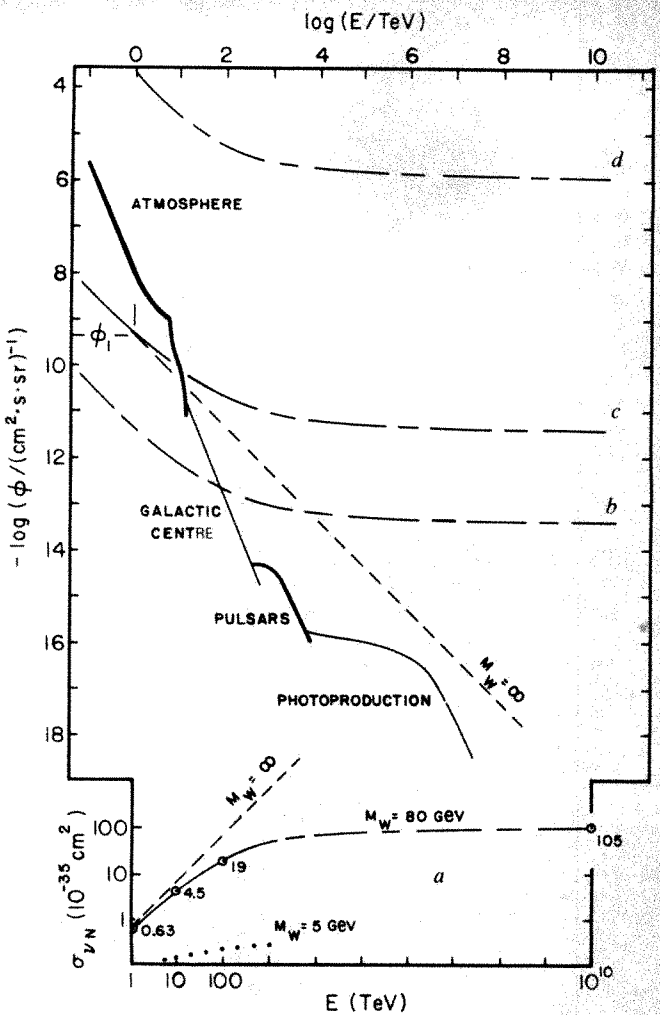


Fig. 3 Natural sources of neutrinos for reconstructive imaging of the Earth's interior density. The neutrino flux ϕ is shown by the solid curves as a function of TeV energy, first compiled in refs 57 and 59. The logarithmic energy scales are equivalent (the upper used in ν-astronomy and the lower in particle physics). The dashed curves are detectors. The flux ϕ_1 at 1 TeV for the 10^9 ton detector is 4.8×10^{-10} cm^{-2} s^{-1} sr^{-1}. Inset a shows the neutrino–nucleon cross-section $\sigma_{\nu N}$ as a function of TeV energy for $M_W = 80$ GeV, and is not a part of the flux diagram. The values indicated at 1, 10 and 100 TeV are taken from ref. 53, while those at 10^3 and 10^4 TeV (35 and 45, respectively) are not shown because the validity of the Buras–Gaemers[56] assumptions in that energy range and beyond is questionable. For $E_\nu > 10^3$ TeV, the curve is dashed to emphasize that it represents only an extension of the Bjorken–Paschos logarithmic term in the Buras–Gaemers–Halprin cross-section at 1, 10, 100 TeV, and is unknown beyond 10^4 TeV. b–d, Detectors capable of measuring 1 count per day and they follow from $\sigma_{\nu N}$ in a and ν-flux arguments given in refs 57–59. b Is 10^{11} tonnes and c is 10^9 tonnes, both of the type considered for DUMAND. d, The flux sensitivity of one orbiting Shuttle external tank (73 trips with 2,157 tonnes of water), as an illustration of the unrealistic effort required for ν-tomography from near-Earth orbit.

and Stecker[59]. The sensitivities of various detectors are derived for $M_W = 80$ GeV, which is essentially an inversion of $\sigma_{\nu N}$ in Fig. 3a to the appropriate flux ϕ. The flux for a 10^9 ton detector at 1 TeV, assuming $M_W = \infty$, is shown as ϕ_1 which calibrated our curve c (Fig. 3), and from which followed curves b and d. We can see from Fig. 3 that a 10^{11} ton DUMAND detector can feasibly measure 100 TeV tomographic neutrinos from the (distended) galactic centre. It seems less probable that a 10^9 ton detector (which measures down to 10 TeV) could differentiate these galactic centre neutrinos from atmospheric, co-rotating neutrinos originating on the opposite side of the Earth and passing through it. (Neutrinos from co-rotating sources, such as the atmosphere and from cosmic rays colliding with the interior, can conceivably be cut from the data using tomographic arguments.) Any other, as yet undiscovered, point source of tomographic neutrinos in the neutrino sky (such as the supernova remnant Cas A or the radio galaxy Cen A, discussed in ref. 57) whose flux lies above the DUMAND detector sensitivity threshold in Fig. 3, might function as well for the 72% core attenuation given in Fig. 2 at 100 TeV.

The flux in Fig. 3 is extremely low (1 event per day), and may be inaccurate by magnitudes. Unless an intense source were discovered in the neutrino sky, the galactic centre represents a varying source I_0 at low flux which means the data (for whole-Earth tomography) must be taken over considerable periods of time. It is feasible but unrealistic.

In conclusion, well-posed mapping schemes require impractical distributions of Tevatrons and neutrino detectors, or detectors over unrealistic periods of time. Whole-Earth tomography

seems out of the question (Fig. 2), requiring inordinate energies (>100 TeV) whose neutrino physics is little understood, although the non-invasive measurement of the density at one or more points in the core is less remote. The true significance of neutrino tomography probably lies not in its implementation but in the importance of where it focuses interdisciplinary attention in geophysics[33] and physics[47–55]: the neutrino cross-sections[47–55] (coherent and incoherent) for constituent substances[33–37] at tomographic energies (10–10^4 TeV), although the neutrinos appear insensitive[55,52] to the coherent nuclei at these energies (as was assumed here). The density at one point in the core or lower mantle is determinable, and such a study is feasible but impractical by this analysis.

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Molecular conformations in surfactant micelles

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For more than half a century, the cornerstone to our understanding of molecular organization in amphiphilic aggregates has been Langmuir's principle of differential solubility[1], according to which the polar heads of amphiphilic molecules dissolve in water whereas the hydrocarbon tails cluster in a core in order to minimize solvent contact[2–5]. This view has recently been challenged; much spectroscopic data[6–17] have been construed[11–17] to indicate significant penetration of water into the cores of micelles. Here various models of micellar structure are critically tested against results from NMR and recent small-angle neutron scattering (SANS) experiments. Molecular organization is found to be the consequence of Langmuir's principle and of the packing requirements imposed by steric constraints among chain molecules at interfaces. The evidence is unambiguous that these cores are virtually devoid of internal water.

SANS experiments[18] were performed on micelles consisting of terminally-deuterated lithium dodecyl sulphate molecules (3.672×10^{-2} M) in 0.3 M LiCl solution in D_2O at 35 °C. The advantages of these conditions for the present purposes are that the micelles are nearly spherical and monodisperse[18–20]. The intensity, $I_D(q)$, of elastically scattered neutrons was measured as a function of wave vector $q=(4\pi/\lambda)\sin(\theta/2)$, where θ is the scattering angle and λ (=4.75 or 5.00 Å) is the wavelength of the neutrons. Parallel experiments were performed on protonated micelles, from which $I_H(q)$ was obtained. The scattering intensity is approximately separable into factors $P(q)$, representing intramicellar structure, of interest here, and $S(q)$, representing the positional correlations of particles in solution; that is, $I(q)=mP(q)S(q)$ where m is the number density of micelles[20]. The difference amplitude, $A(q)$, of the scattering from the chain termini can be calculated from the measured intensities by

$$A(q)=\{[I_H(q)]^{1/2}-[I_D(q)]^{1/2}\}[S(q)]^{-1/2} \qquad (1)$$

the procedure for calculating $S(q)$ is presented elsewhere[18–20]. Values for $A(q)$ thus obtained from our experiments are shown in Fig. 1. The radial density distribution, $N_{Me}(r)$, of termini per unit volume throughout the micellar core can be derived from $A(q)$ (ref. 18)

$$A(q)=m^{1/2}\int_0^\infty 4\pi r^2 b_{Me}N_{Me}(r)\left(\frac{\sin qr}{qr}\right)dr \qquad (2)$$

where $b_{Me}=|b_{CH3}-b_{CD3}|=3.12\times10^{-12}$ cm is the difference in scattering lengths.

Various models of micellar core structure predict different radial density distributions, $N_{Me}(r)$, of the termini therein. Evaluation of equation (2) according to each model described below yields the set of scattering functions $A(q)$ shown in Fig. 1. To calculate these distributions, it is convenient to consider the hydrocarbon core as being partitioned into discrete concentric shells, numbered $i=1,2,3\ldots,n+1$, towards the centre. Chains are assumed to have $n+1$ segments. The ith shell contains $N_i=(4\pi/3)[3(n+2-i)(n+1-i)+1]$ equivolume sites. Each site contains at most one chain segment. For chain lengths to be independent of configuration, sites must be approximately isodiametric[21,22]; dodecyl chains are thus most suitably

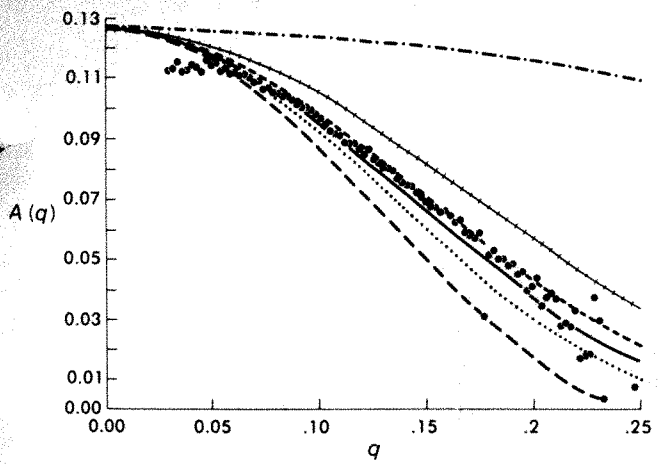


Fig. 1 Small-angle neutron scattering amplitudes, $A(q)$ against wavevector, q. Data from ref. 18, corrected here for interparticle interactions according to equation (1). Predictions (radial shells are numbered $i = 1, 2, 3, 4$, towards the micellar centre) are given by: (1) Radial chain model; $t_i = 0, 0, 0, 1$ (—·—). (2) Pincushion model; $t_i = 0, 0.563, 0.375, 0.062$ (++++). (3) Interphase model, $\omega = 1$; $t_i = 0.234, 0.414, 0.289, 0.062$ (– – –). (4) Interphase model, $\omega = 0.5$; $t_i = 0.326, 0.370, 0.241, 0.062$ (——). (5) Interphase model, $\omega \to 0$ (identical to Cabane model[25]); $t_i = 0.438, 0.312, 0.188, 0.062$ (· · · · ·). (6) Oil droplet model; $t_i = 0.578, 0.297, 0.109, 0.016$ (— —).

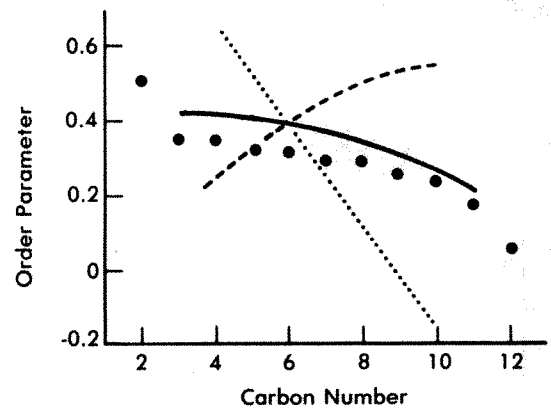


Fig. 2 ^{2}H-NMR disorder gradient measurements of potassium laurate micelles in the hexagonal phase against chain position from the head group. Data from ref. 27 are multipled by 2 to correspond to the bond order parameter defined according to the interphase theory[22,31]: – – –, freely flexible chains, $\omega = 1$; ——, 'real' chains, $\omega = 0.5$; · · · · · rigid molecules, $\omega \to 0$. The oil droplet model predicts $S = 0$ and the radial chain model $S = 1$ for all segments.

represented by $n+1 \cong 4$. The end group density may be expressed as $N_{Me}(r) = (J_1/N_i) \cdot [(n+1)/R]^3 \cdot t_i$, where t_i is the fraction of chains whose termini are in layer i, $J_1 = 82$ is the number of chains per micelle, and $R = 18.9$ Å is the equivalent spherical radius of the micellar core[19].

The following models are considered: (1) The radial chain model. Micellar chains are often depicted as radially aligned and largely all-*trans*[23,24]. Taken literally, this model would require all termini to occupy the same element of volume at the core centre, in violation of volume exclusion constraints. That this model, which represents the maximum possible radial order and porosity of the core to solvent, is not viable is evident from comparison with the SANS and other results (see Fig. 1 and refs 25, 26).

(2) The pincushion model[11] admits of lesser—but significant—water penetration into outer regions of the core. For purposes of calculation, we adopt a version of this model according to which chain ends are distributed as close to the core centre as is consistent with packing constraints. The core centre contains only termini. Sites in the surrounding shell are occupied by the few radially-aligned bonds required to connect to the centre termini and are otherwise filled by chain termini: this shell contains no lateral bonds (that is, those normal to the radius). The next (third) shell is similarly occupied by forward steps to central layers and by the remaining termini. This and surrounding shells may be further occupied by any distribution of lateral and forward segments and solvent molecules with no further bearing on the distribution of termini. Thus, this model represents a class of configurations with water penetration of 20–60% of the outer layers of the core. This model is also in poor accord with the SANS data (Fig. 1).

(3) In the oil droplet model[3], the micellar core resembles pure, amorphous, liquid alkane. Accordingly, chain ends are distributed uniformly throughout the core, the number in each shell being proportional to its volume. This model overestimates the methyl density at the solvent interface (see Fig. 1 and ref. 18). Other evidence shows that the chains in micellar cores are more ordered than in liquid alkanes[1,3,25–30].

(4) According to the interphase model[21,22,31,32], the core is filled exclusively by chain segments which are randomly distributed except as required to preserve chain continuity and satisfy excluded volume constraints. These requirements are taken into account through application of a matrix method[21,22,31]. The first chain segments connected to head groups are required to occupy the outermost shell of the core; the remaining sites of that shell are filled by laterally bonded segments. Other layers are similarly occupied by bonds which enter shell i from $i-1$, or by lateral bonds in i. Subject to these constraints, chain segment orientation is otherwise random, for freely flexible chains[21,22]. Real chains are not freely flexible, however. Their stiffness may be taken approximately into account through a statistical weight ω, representing the *a priori* probability of a 'bent' bond pair relative to a co-linear pair in an isolated molecule[31,32]. With this refinement, freely flexible chains are represented by $\omega = 1$, rigid molecules by $\omega \to 0$ and real polymethylene chains at $T = 300$ K approximately by $0.4 \leq \omega \leq 0.5$; predictions for these three cases are shown in Fig. 1. Since the interphase model requires complete filling of the core volume, then even for $\omega \to 0$, most chains are required to have one bend; in this limit, the chain configurations are identical to those predicted by the simple micellar packing model of Cabane[25].

The results of the interphase model have been mimicked through an alternative formulation[33,34] in which intramolecular interactions are treated in the rotational isomeric state approximation[35], but in which steric packing constraints are represented through internal pressures which differ in each shell. The latter premise is unsatisfactory inasmuch as the requirement for mechanical equilibrium is thereby violated; these pressures are introduced to simulate packing constraints. The constraints are modelled explicitly, however, by the interphase treatment described above.

Interphase theory predictions are in good agreement with experimental measurements of configurational properties of amphiphilic aggregates[1,21,22,26,32,36]. We have previously noted that one prediction, however, has been in disagreement with experiment[1]; here that issue is resolved. The interphase theory for freely flexible chains predicts that the order should increase towards the chain ends in micelles (see the dashed curve in Fig. 2; see also ref. 22). ^{2}H-NMR experiments with perdeuterated chains in hexagonal phase micelles[27] show the reverse, that there is increasing orientational disorder towards the terminal groups. This closely resembles the 'disorder gradient' in bilayer membranes[21,37]. Recent refinement of interphase theory[31,32] shows that orientational order in micelles depends markedly on chain stiffness, neglected in the earlier approximation[22] (compare the solid curve in Fig. 2). Intramolecular interactions thus contribute significantly to micellar chain organization whereas for planar bilayers the disorder gradient depends primarily on intermolecular constraints[32].

There is broad support for the view that water is largely excluded from the interior of micellar hydrocarbon cores (refs 1, 3, 4, 18, 25 and references therein). This conclusion is corrob-

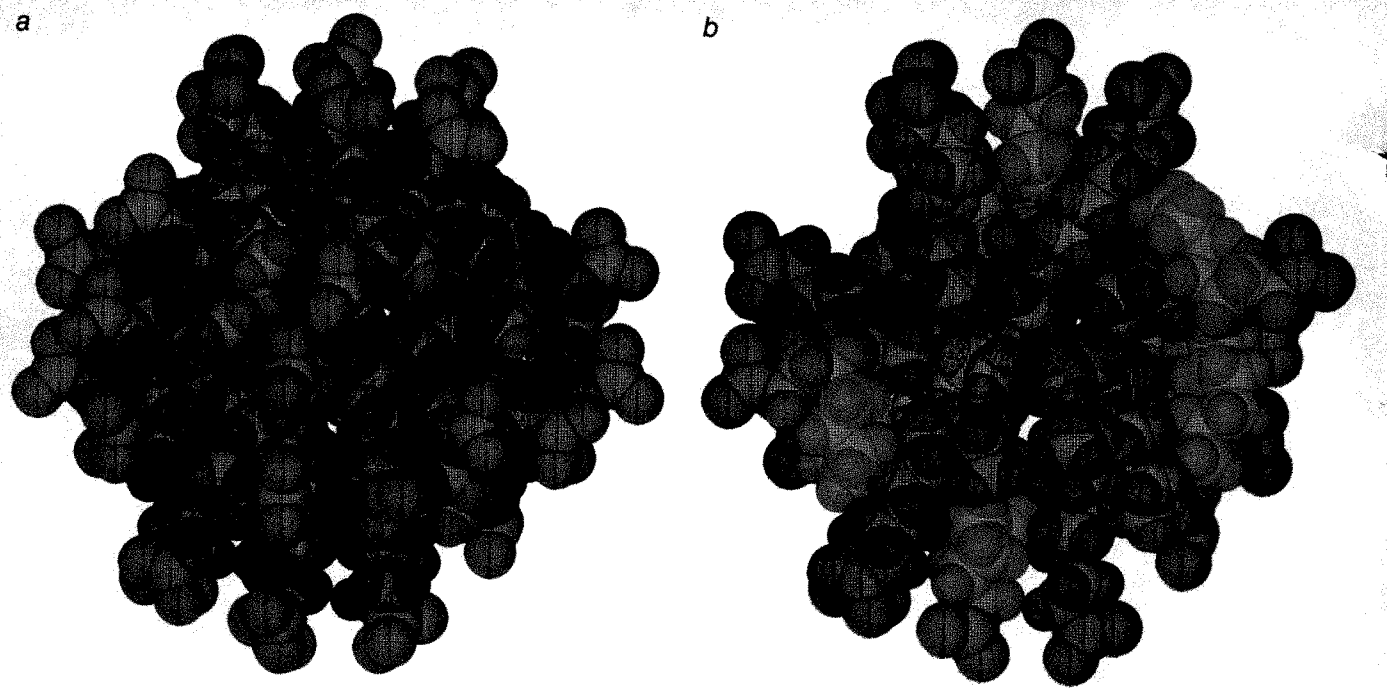


Fig. 3 Representative configuration of a 38-chain spherical decanoate micelle according to the interphase theory[22,31]. Computer graphics were performed with a modified version of SPACFIL at Molecular Design, Ltd. The micelle was constructed by configuring chains on a spherical lattice, then applying a nonlinear least-squares method to best fit vectors, representing C–C bonds between methylenes, through the appropriate lattice sites. *a*, Outside surface. Head groups in green, methylenes in black and terminal methyls in red. Much hydrocarbon, including some chain ends, is exposed to solvent. *b*, Micellar interior; front hemisphere removed. Red molecules terminate near the surface, blue ones midway to the centre and black ones at the core centre. In this view, chain disorder, distribution of termini and density of packing are evident.

orated by the results of Fig. 1 showing that the differential SANS data are best modelled by dry hydrocarbon core configurations. The strongest support for this view, however, comes fron SANS contrast variation measurements of absolute scattering length density[18,38]. In a series of independent measurements[18], lithium dodecyl sulphate micelles with mean aggregation number of 78 were shown to have a hydrocarbon core volume per molecule ($28{,}085/78 = 360$ Å^3) equal to that of a pure dodecyl chain[3]. Moreover, the measured scattering length density of the core ($\rho = -3.8\times10^{-7}$ Å^{-2}) is exactly equal to that predicted for a dry core consisting exclusively of dodecyl chains ($b_{C12} = -1.37\times10^{-12}$ cm; thus $\rho = -1.37\times10^{-4}$ Å$/360$ Å$^3 = -3.8\times10^{-7}$ Å^{-2}). These experiments could have readily detected one D_2O molecule per amphiphile in the core. X-ray diffraction experiments[39] are more equivocal because they only provide relative densities. The preponderance of support for the alternative view[11–17], that there is water penetration, has come from spectroscopic experiments, in which water-insoluble probes in micellar solution have been found to reside in a partly aqueous environment[6–17,40,41]. The two alternative interpretations of these data are that the probes and water may associate either in the core or at the interface. Recent ring-current shift NMR measurements show that many of these probes reside at the interface[40–42]. ^{19}F-NMR spectroscopy on perfluorooctanoate micelles shows that the segments of the micellar chains similarly reside in a partly aqueous environment[43]. This result should not be surprising, however, for nearly 60% of the chain segments in small micelles are at the surface[1]. Recent interphase theory calculations predict quantitatively the degree of water contact of each of the chain segments as measured in the ^{19}F-NMR experiments[1]; it is the consequence of the high equilibrium incidence of chain segments at the core interface. We conclude that micellar hydrocarbon cores are virtually devoid of internal water, but that substantial hydrocarbon/water contact occurs at the core interface.

Computer graphics representations of the interphase model (Fig. 3*a*, *b*) show the degree of chain disorder, the distribution of termini and the extent to which hydrocarbon segments, including chain ends, are exposed to the solvent at the core interface.

The structures and properties of micellar aggregates are thus found to be the consequences of: (1) Langmuir's principle of differential solubility, according to which hydrocarbons are sequestered into a core devoid of water surrounded by the polar heads; (2) the principle that steric forces predominantly determine the structures of condensed phases[44]; and (3) the statistical mechanical principle that degrees of freedom of equal energy (in this case, of the intramolecular chain configurations) are of equal likelihood.

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Note added in proof: In a recent study[45], it is claimed that micellar chains form 'loops', with termini 200 times more exposed to solvent than internal segments. Those results have no bearing on unperturbed micelles, however, for the SANS results presented here would have detected even a small degree of looping, and showed none. In that study, termini and internal groups were double bonds on probe molecules of significantly different lengths. Neither the locations of those molecules nor their double bonds with respect to any micelles present were known. Double bonds are attracted to water[3] and chains of different lengths prefer different phases; few extrinsic probes of micelles are innocuous[1,4].

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Synchrotron X-ray diffraction from a 800 μm^3 zeolite microcrystal

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The brightness of synchrotron X-ray sources, which exceeds that available from conventional generators by many orders of magnitude[1–3], provides the possibility of making conventional measurements on much smaller samples. We describe here measurements of Bragg diffracted intensities from two microcrystals of cancrinite, a typical zeolite, performed in order to assess the scope of microcrystal diffraction. Our results demonstrate that many materials previously regarded as polycrystalline can now be studied using well established single-crystal methods at synchrotron facilities.

Crystals of basic/nitrate cancrinite[4–7] were selected from a hydrothermal crystallization batch (F. W. Webster and E. A. D. White, unpublished) that contained specimens ranging in size from several micrometres up to millimetres. Cancrinite is a typical zeolite with an open structure built of atoms of low atomic number [actual composition in this case $Na_6Al_6Si_6O_{24}$. $0.33(NaNO_3)$. $1.22(NaOH)$. $2.2(H_2O)$] and with some structural imperfection. It therefore provides a near 'worst case' test. One of the larger crystals from the batch had been measured on a conventional CAD-4F diffractometer[8], enabling us to make a direct correlation between measured intensities and structure factors of known magnitude. The cancrinite structure has hexagonal symmetry and the crystals generally had needle-like morphologies, the long direction coinciding with the unique crystallographic axis. This elongate characteristic allowed the 001 direction to be defined visually in an optical microscope, the selected crystals being too small to align by conventional X-ray photographic techniques. Two crystals of approximate dimensions $35 \times 25 \times 25$ μm (no. 1) and $20 \times 10 \times 4$ μm (no. 2) (dimensions estimated by reference to a 12.5-μm eyepiece scale) were selected, glued to the ends of Pyrex glass fibres of ~10 μm outside diameter, cut to 1 cm long, mounted on eucentric goniometer arcs and aligned so as to rotate about a direction perpendicular to the long axis.

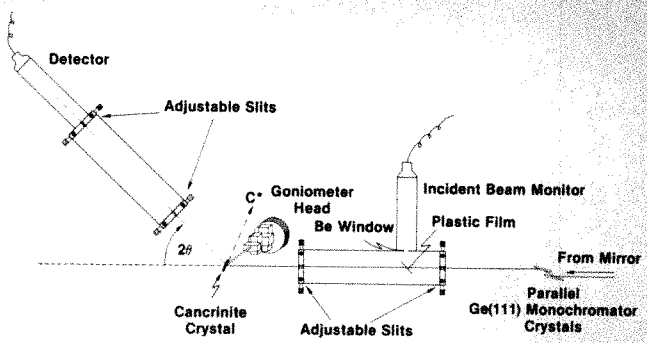


Fig. 1 Schematic diagram of the experimental arrangement. The evacuated boxes housing the adjustable slits and the beam monitor arrangement are shown. Because of the beam polarization, the scattering plane is vertical and the crystal orientation was chosen such that the c^* axis would lie in this plane at $\chi \approx 0°$ (as indicated).

Measurements were made on the 4-circle Huber diffractometer located at the VII-2 Wiggler line at the Stanford Synchrotron Radiation Laboratory, Stanford[9], during the tune-up period after the summer shutdown, so that the beam condition was rather poor (see below). On the VII-2 beam line, 4.6 mrad of arc can be viewed by the mirror which focuses the beam at the sample position. After the mirror, a mean incident wavelength of 1.74 Å was selected from a pair of parallel, asymmetrically cut Ge(111) crystals. Both incident and diffracted beams were collimated by evacuated adjustable slit systems (Fig. 1). The arcs with the cancrinite crystals were mounted directly onto the diffractometer, the only adjustment made being that of sample height. No particular precautions were taken to minimize the background.

At the chosen wavelength, we had access to two significant $00l$ reflections, $l = 2$ and $l = 4$. The configuration was chosen so that the c^* axis would lie in the scattering (vertical) plane (Fig. 1). The crystals were rotated about the spindle axis with the detector set at the 002 position until significant intensity was observed in the counter meter. The reflections were then centred incrementally in each of the circles. In both cases, only minor adjustment of the χ-circle was necessary, demonstrating the effectiveness of the initial optical alignment procedure. Table 1 summarizes the data obtained in radial and rocking-curve scans of the crystals, while scans of the smaller crystal are plotted in Fig. 2. For the larger crystal (no. 1), we also made a scan along $00l$ from $l = 1.990$ to 3.015 which indicated that both satellites and the 003 reflection have structure factors of less than 0.9 electrons.

The total energy, E, reflected by a crystal volume δV as it is rotated through the region of a Bragg reflection hkl is (see, for example, ref. 10)

$$E = I_0 \left(\frac{e^2}{4\pi\varepsilon_0 mc^2}\right)^2 \frac{\lambda^3}{V^2} \delta V |F_{hkl}|^2 \frac{1}{\sin 2\theta} \frac{1}{\omega}$$

We have used this expression to estimate I_0, the incident photon flux within the resolution element sampled, from the integrated intensities given in Table 1. The results are given in Table 2. Considering the poor beam conditions, the errors associated with estimating E (Table 2) and the crystal dimensions (as above), the agreement between the three independent estimates is reasonable.

The measured background levels were low. For example, at $h = 0$, $k = 0$, $l = 2.1$ (well away from the central Bragg point), the backgrounds were 2.4 (no. 1) and 0.7 (no. 2) counts s^{-1}, respectively. On the edge of the wings of the Bragg peaks, three halfwidths from the central point, the observed signals were 8.5

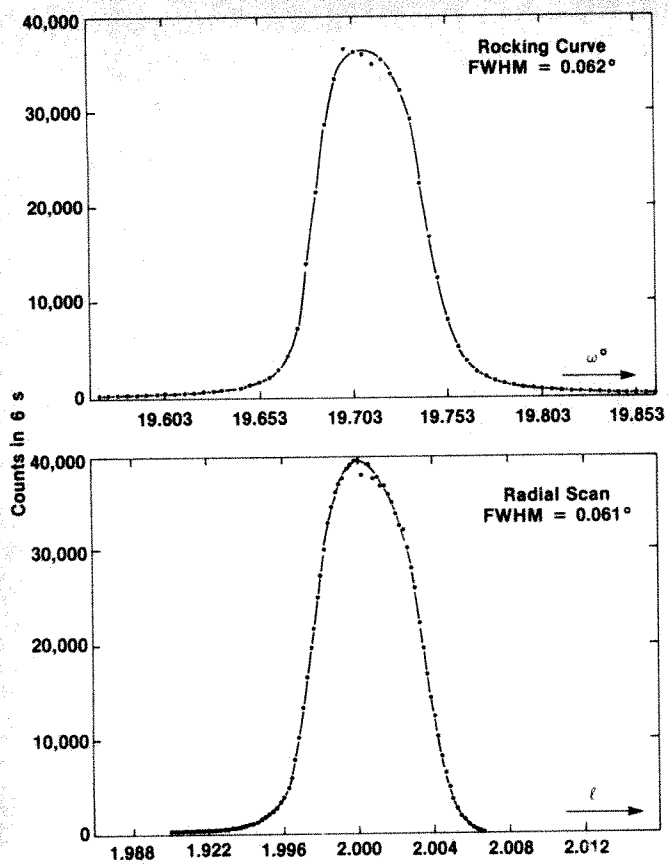


Fig. 2 Scans through the 002 reflection from the 800-μm^3 cancrinite crystal (no. 2). The upper, rocking curve indicates the mosaic spread of the crystal.

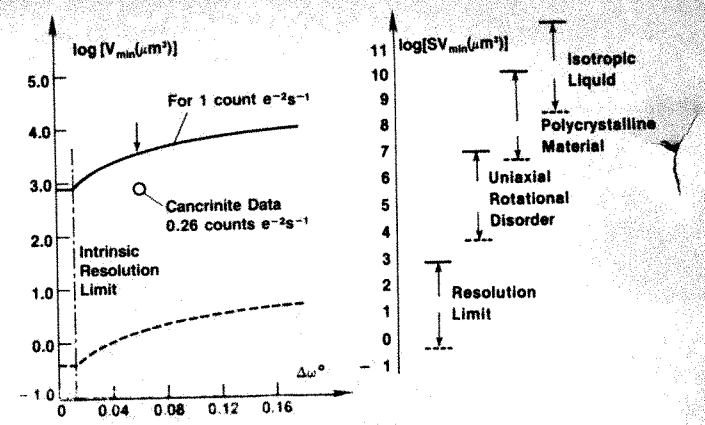


Fig. 3 The minimum crystal volume, V_{min}, that is required to provide a peak count rate of 1 count per electron^2 per s plotted against the rocking curve width or crystal mosaicity, $\Delta\omega^0$. The 800-μm^3 cancrinite crystal provided 0.26 counts per electron^2 per s (marked). The upper (solid) lines refer to the conditions under which the present measurements were made. The lower (dashed) lines represent the performance expected if an anticipated gain in flux of 2×10^3 is realized (see text). Extrapolation of these curves to $\Delta\omega = 180°$ (corresponding to uniaxial rotational disorder), to peak broadening in the second dimension (representing a random collection of crystallites or 'powder' sample), and finally to peak broadening in 2θ space permit the estimation of the minimum sample volumes (SV_{min}) required to provide 1 count per electron^2 per s at peak from other types of sample (right-hand portion of figure).

(no. 1) and 6 (no. 2) counts s^{-1}. We believe that the background levels could be further reduced by improved crystal mountings, optimized collimation and use of evacuated or helium flight paths. (Background rates as low as 0.1 counts s^{-1} have been achieved in similar experiments on this beam line.)

The size of the smallest measurable crystal will depend on the structure factor (SF) and the counting time allowed to reach a specified signal-to-noise ratio. We will adopt, for illustrative purposes, a very weak SF of 10 electrons, and 100s to achieve 1% statistical accuracy at the peak position. Since in 100 s the background (at 0.1 counts s^{-1}) is expected to be only 10 counts, it can be ignored but larger background rates can obviously increase the minimum measurable volume. In our experiment we recorded equivalent to 0.26 counts per electron^2 per s or one-quarter of the required accuracy. A complete data set of high precision could thus have been obtained from a 3×10^3 μm^3 crystal in the conditions of the present experiment.

In optimized beam line conditions, use of a shorter X-ray wavelength and the new Wiggler beam line, one would obtain an enhancement of flux of 2×10^3–2×10^4, with a concomitant reduction in the minimum measurable crystal volume. Thus, in these optimum conditions, complete high-precision data sets could be measured from crystals having volumes less than 1 μm^3.

It is the brightness of the synchrotron source, the incident

Table 1 Data from radial and rocking-curve scans of crystals

Scan no.	Crystal	Reflection	Type	Background*	Peak*	Intensity*	FWHM (deg)
1	1	002	$\omega-2\theta$	3	1.1×10^5	8.0×10^6	0.0686
3	1	002	ω	3	1.1×10^5	1.8×10^6	0.0687
5	1	004	$\omega-2\theta$	3	7.2×10^3	7.8×10^4	0.1412
6	1	004	ω	3	7.2×10^3	1.2×10^5	0.0736
8	2	002	$\omega-2\theta$	4	6.9×10^3	2.1×10^5	0.0605
9	2	002	ω	4	6.2×10^3	8.4×10^4	0.0620

* Normalized to counts s^{-1}.

Table 2 Estimation of total energy E and incident photon flux I_0 for cancrinite crystals

Crystal no.	Crystal volume	Reflection	F_{hkl}	ω(rad s^{-1})	E	I_0
1	$13{,}000\times10^{-18}$	002	165	2.424×10^{-5}	9.55×10^6	5.24×10^{15}
1	$13{,}000\times10^{-18}$	004	105	1.897×10^{-5}	1.53×10^6	2.54×10^{15}
2	800×10^{-18}	002	165	1.505×10^{-5}	6.36×10^5	3.53×10^{15}

Cancrinite unit cell, $a = 12.69$, $c = 5.16$ Å, $V = 727$ Å^3, space group $P6_3$. The structure factor in electrons, F_{hkl}, was taken to be that measured from a much larger crystal of the same composition on a CAD-4F diffractometer[8]. E is calculated as $I_\omega \cdot f_a$, where I_ω is the integrated intensity recorded in the ω-scan (normalized to the same beam current as the first scan) and f_a is an estimated correction for the diffracted beam collimation in 2θ space (defined as the ratio of the total area of the gaussian of halfwidth FWHM (Table 1) to the area of the gaussian encompassed by the collimation).

photon flux per resolution element, that is the key parameter in this application. At the optimum, the sample mosaic width would match the intrinsically high resolution of the source, but for the crystals studied here, which we believe to be fairly typical, the mosaic width (0.062°) is four times greater than the inherent beam divergence (0.014°). The narrowing in the peak width that would accompany an improvement in crystal quality would enhance the peak count rate and hence further reduce the minimum required sample volume (Fig. 3). To push the technique to its size limit, crystals with a high degree of perfection will be required, although in this size regime one might expect that the smaller the crystal size, the greater the likelihood of crystal perfection.

In many fields great importance is attached to structural characterization and to proper interpretation of the interplay between structure and properties. Although single-crystal diffraction data are generally required for structure solution and detailed refinement, many materials do not occur in suitable single-crystal form. This has prompted widespread interest in the development of techniques for using powder data in optimizing structures that are already approximately known[11]. With powder data, particle-size broadening effects generally become apparent for average particle dimensions less than 0.2 μm. Our present results indicate that polycrystalline samples in which such effects are not observed can in many cases be regarded as large selections of possible candidates for structure solution and analysis using single-crystal techniques at synchrotron facilities. *Note added in proof*: Bachmann *et al.*[12] recently described measurements on a CaF_2 microcrystal at DESY. Their conclusions are similar to those presented here.

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Propagated temperature changes during onset and recovery of the 1982–83 El Niño

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El Niño is the appearance of anomalously warm sea surface temperatures along the coast of Peru. El Niño is also associated with changes of sea level and thermocline depth which are linked to a large-scale interaction of the global atmosphere and tropical ocean[1–4]. Linear wind driven theory suggests that Kelvin waves are responsible for the El Niño thermocline displacements[3], but in model systems it is difficult to relate thermocline changes induced by Kelvin waves to the observed changes in sea surface temperature[5]. We have now examined daily sea surface temperatures along the coast of Peru and subsurface temperature records from the Galapagos and 10° S and 79° W (Fig. 1) made during onset and recovery of the 1982–83 El Niño[6]. Our data show that the onset of the anomalous warming in this region propagated at speeds slower than Kelvin wave speeds. The speed of travel calculated from changes in temperature from the Galapagos to the coast of South America and then poleward along the coast is extremely coherent, and during onset appears to be related to advective processes. The rapid decrease in sea surface temperature associated with recovery from the 1982–83 El Niño was propagated at much higher speeds, very suggestive of Kelvin wave dynamics.

In 1894 Eguiguren[7] noted that there was a correlation between the rainy years in northern Peru and warm ocean temperatures associated with the years in which the El Niño current was of unusual strength. Half a century later Bjerknes[8] linked these episodes of warm sea surface temperatures (SSTs) to large-scale meteorological events associated with the Southern Oscillation[1–4]. Recently Wyrtki[2] has argued that El Niño is a dynamic response of the entire Pacific basin to changes in large-scale wind forcing; the response of the ocean includes changes in sea level, thermocline depth and horizontal advection.

Observations have shown that changes in sea level propagate rapidly from the western Pacific to the east along the Equator and then poleward along the coast of Peru[9,10]. Linear wind driven theory suggests that these changes result from packets of Kelvin waves that are excited by anomalies in the trade wind field in the western and central Pacific[3]. Theory also suggests that Kelvin waves are responsible for the El Niño thermocline displacements. Analysis of the daily temperature changes in 1982 and 1983 conntribute to a description of the dynamic characteristics of El Niño and provide an interesting complement to the theory.

Temperature observations made from piers at Paita (5° S), Lobos de Afuera island (7° S), Pimentel (7° S), Supe (11° S) and Callao (12° S) are shown in Fig. 2. The Paita measurements are made at 0800 h daily while the others are averages of measurements made at 0600, 1200 and 1800 h daily. The Paita, Lobos de Afuera and Callao time series (Fig. 2*a*) show that the initiation of the temperature rise at these locations occurred on 23 September, 29 September and 18 October respectively. The speed of propagation of these temperature rises, assuming straight trajectories is 43.6 cm s^{-1} between Paita and Lobos de Afuera and 42.3 cm s^{-1} between Lobos and Callao (Table 1). Smith[11] reported that temperature measurements from a depth of 100 m on a current meter array located over the 150 m isobath

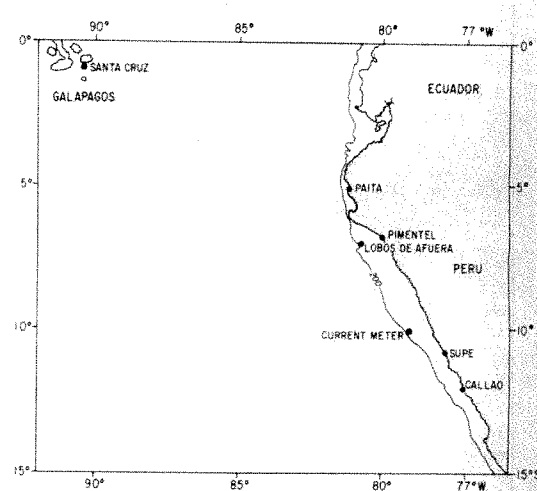


Fig. 1 Chart of the eastern equatorial Pacific showing the locations that provided observations for this article. The 200 fathom contour is included to show the position of the shelf break.

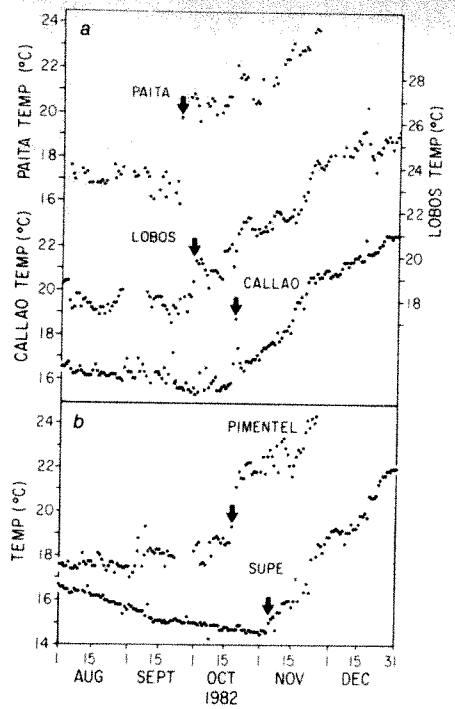


Fig. 2 Daily sea surface temperature observations made from piers at *a*, Paita (5° S), Lobos de Afuera (7° S) and Callao (12° S); and *b*, Pimentel (7° S) and Supe (11° S) during onset of the 1982–83 El Niño. Arrows indicate beginning of the temperature rise used to calculate propagation speeds.

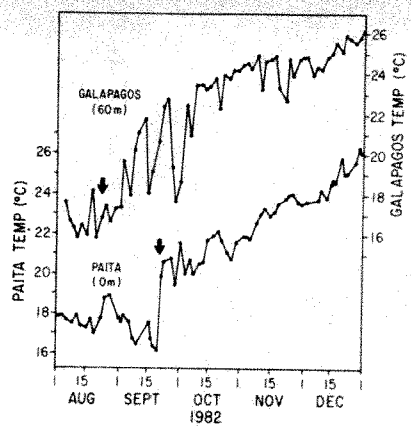


Fig. 3 Tri-weekly temperature observations from 60 m at Paita (5°05′ S, 81°16′ W) and Puerto Ayora, Santa Cruz Island, Galapagos Islands (0°47′ S, 90°17′ W). These locations are over the 100 m isobath. Arrows indicate beginning of the temperature rise used to calculate propagation speeds.

at 10° S showed the beginning of a change in temperature on 7 October. The temperature rise at the surface at a station offshore of Paita which is occupied tri-weekly over the 100 m isobath was first detected on 22 September (no observation made on 21 September). The propagating speed between the Paita offshore station and the 100 m current meter at 10° S is either 43.9 or 46.8 cm s^{-1}. Interestingly the poleward current at 100 m increased in speed from 19 to 36 cm s^{-1} between 6 and 7 October[11], a velocity similar to our calculations of the speed at which the onset of the temperature increase propagated.

Figure 3 shows the temperature time series from the Paita offshore station at 5° S and 81°17′ W and a parallel station maintained at Santa Cruz island at 0°47′ S and 90°17′ W in the Galapagos. The beginning of the temperature rise at 60 m in the Galapagos occurs on 23 August, in agreement with other reports from the islands that warming there began in mid August[12]. Assuming a straight line path directly from Santa Cruz to Paita (Fig. 1), the speed of travel of the temperature rise is 42.4 cm s^{-1}; assuming a maximum distance path along the Equator to the coast and then poleward down the coast the speed of travel is 60 cm s^{-1}, a speed used to describe the eastward propagation of the equatorial zonal SST gradient[13].

A second feature of the SST records from the Peru coast is that the increase in temperature at Pimentel (7° S) and Supe (11° S) (Fig. 2*b*) lag the other coastal stations. The calculated speed of travel from Lobos de Afuera to Pimentel (Fig. 1) is 5.1 cm s^{-1}. Mean onshore currents of 5.0 cm s^{-1} have been measured on the coast of Peru[14]; this velocity is surprisingly close to the calculated speed for the cross-shelf travel from Lobos de Afuera to Pimentel. Upwelling favourable winds along the coast of Peru remained strong throughout December 1982[11], so it is appropriate to assume that a typical cross-shelf upwelling circulation was maintained. Additional evidence for cross-shelf propagation of the temperature changes comes from the Paita offshore station which is 7 km from the coast. At the station the rise led the rise at the Paita pier; however, the tri-weekly sampling does not permit a precise calculation of travel speed over this short distance.

The lag in the temperature rise at Supe (11° S) is difficult to account for. If we assume that a route directly across the shelf from the 200 fathom contour (Fig. 1) then the speed of travel of the rise is calculated to be 4.6 cm s^{-1}. However, there is no reason to restrict the poleward undercurrent to the shelf break as observations during 1977[15] showed the undercurrent was on the shelf at 10° S and 12° S. The lag observed at Supe suggests that the local circulation must have shielded this location.

The mean SST field for this region in September[16] shows that there is a relatively small offshore gradient of SST (17 to 19° C in 100 km) so anomalous onshore surface transport would not result in a significant temperature signal, but there is a strong (17 to 25° C in 100 km) gradient in the alongshore dimension equatorward of Paita. The September 1982 rise in temperature at Paita shows an increase in temperature in a period of a few weeks which is similar to the mean alongshore gradient in that region. Large-scale horizontal temperature distributions[17] for the latter part of 1982 do not show significant changes until November, well after the temperature increases were observed at the shore stations. Our interpretation of this large-scale temperature increase in November 1982 is that it results from movement of the equatorial front southward; temperature, salinity and nutrient observations[18] support this interpretation.

The speed and spatial extent of the decrease in SST and subsurface temperatures associated with recovery from the

Table 1 Travel speeds of the appearance of the anomalous warming between locations shown in Fig. 1

From	On	To	On	Distance (km)	Time (days)	Speed (km day^{-1})	Speed (cm s^{-1})
Paita pier	23 September	Lobos pier	29 September	226	6	37.7	43.6
Lobos pier	29 September	Callao pier	18 October	695	19	36.5	42.3
Paita offshore	21 September	Current meter	7 October	607	16	37.9	43.9
	22 September	(100 m)	7 October	607	15	40.4	46.8
Santa Cruz (60 m)	23 August	Paita offshore	22 September	1,100	30	36.7	42.4
Lobos pier	29 September	Pimentel pier	18 October	83	19	4.4	5.1

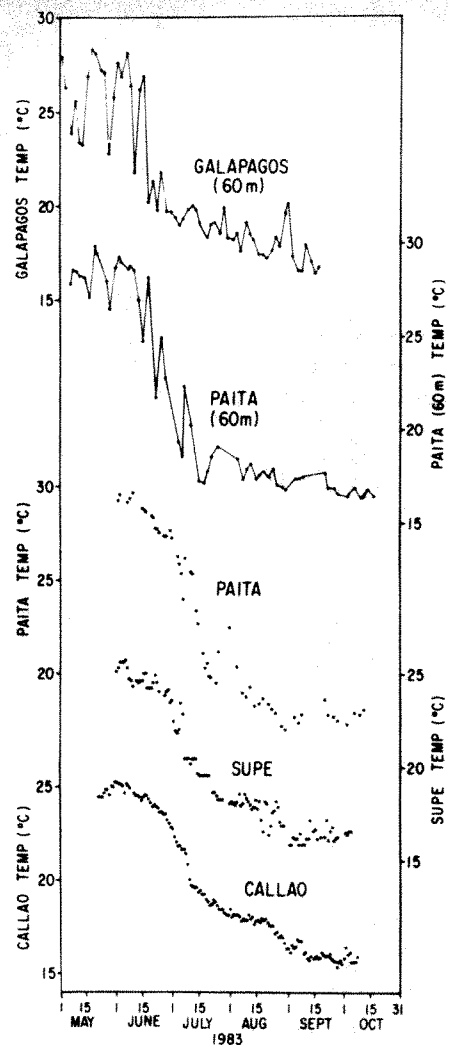


Fig. 4 Tri-weekly temperature observations from 60 m at Paita (5°05′ S, 81°16′ W) and Puerto Ayora, Santa Cruz Island, Galapagos Islands (0°47′ S, 90°17′ W) and daily sea surface temperature observations made from piers at Paita (5° S), Supe (11° S), and Callao (12° S) during recovery of the 1982–83 El Niño.

1982–83 El Niño are impressive. Figure 4 shows time series of temperatures from Galapagos (60 m), Paita (60 m), Paita, Supe and Callao during the recovery. Temperatures at 60 m in the Galapagos and Paita and SST from Paita, Supe and Callao drop almost simultaneously. From Fig. 4 it appears that recovery at 60 m in the Galapagos leads the Peru coast recovery and that recovery at 60 m at Paita leads the surface recovery at Paita. From 15 to 17 June there is a 6 °C temperature drop at 60 m at the Galapagos, and a similar drop (6 °C) is observed at the Paita 60 m station between the 17 and 22 June. Assuming a minimum distance path a propagating speed of 254 cm s^{-1} is calculated; assuming a maximum distance path following the coast and Equator the speed is 345 cm s^{-1}. This velocity domain is suggestive of Kelvin wave dynamics.

The 1982–83 El Niño differed from the canonical event described by Rasmusson and Carpenter[4] in that onset was some 6 months later in the year, so that during 1982 the anomalies in temperature along the coast of Peru appeared in September as opposed to February; this is important because the temperature fields off the Peru coast during September and February[16] are very different. The remotely forced perturbation in 1982 was, therefore, 'received' by a different thermal regime than the canonical event. Analysis of the day-to-day temperatures changes from previous El Niño events may help elucidate the mechanisms responsible for El Niño SST anomalies.

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A wetter climate in eastern Sudan 2,000 years ago?

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Within the past 15 years accurate radiocarbon dating and plant microfossil analysis of tropical lake sediments in Africa and elsewhere have revealed that the early Holocene (11–7 kyr) was generally wetter than today throughout the tropics[1–4], in contrast to last glacial maximum (18 ± 2 kyr), which was cool, dry and windy[5,6]. Desiccation beginning ~4.5 kyr ago forced Neolithic herders to abandon previously habitable Old World deserts[7–9]. Less well known is the climate of proto-historic times (~2 kyr ago), when iron-smelting became important in Africa[10]. Here we present the first evidence that the Red Sea Hills were somewhat wetter at this time and suggest that this was also true of much of northern Africa.

Prompted by an early report of sub-fossil land and pond snails at Erkowit[11] in the arid Red Sea Hills of Sudan, we have collected, identified and dated freshwater molluscs from the alluvial clays which line the main valley near the original site. The mollusc and ostracod assemblage in all our samples is consistent with perennial stream flow some 2,000 years ago in an area now devoid of permanent rivers. The Erkowit plateau lies at 18°5′ N, 37°10′ E in the Red Sea Hills of eastern Sudan (Fig. 1). It rises over 1,200 m above the Red Sea, from which come frequent winter mists that help sustain sporadic *Euphorbia candelabra* and other evergreen Ethiopian upland plants[12,13]. Annual rainfall at Erkowit is ~300 mm, with twin peaks in January and August[14,15]. The surrounding country is much drier[15].

Up to 6 m of shell-bearing, alluvial clays are exposed in ephemeral stream channels like Khor Harasab where they overlie coarse angular gravels (Fig. 2). In nearby tributary gullies the clays sit unconformably on Basement Complex bedrock. Mantling the clays are sands up to 2 m thick, which are still being laid down. Over-grazing has led to gully erosion since the 1920s[11], with exposure of the fossiliferous clays.

In addition to the freshwater molluscs *Melanoides tuberculata* and *Bulinus truncatus*, both of which are common in the White Nile, Tothill[11] collected two other species of aquatic molluscs from the Erkowit clays (Table 1*a*). We have identified five species of aquatic snails scattered through the clays (Fig. 2 and Table 1*b*), but no land snails, in contrast to Tothill, who collected further from the valley bottom[11]. The ostracod assemblage

found in association with the freshwater mollusc assemblage (Fig. 2) indicates that the clays were laid down in permanent water.

We found shells scattered through the upper 4 m of alluvium, with rather more *Melanoides* and *Biomphalaria* near the top and bottom of the deposit (Fig. 2). The two ^{14}C dates were from these two levels, and do not differ significantly in age at one standard deviation. After cleaning in an ultrasonic bath and etching with dilute hydrochloric acid, SUA-1794 was counted for 1,760 min, and SUA-1795 for 5,000 min. Only *Melanoides* shells were dated. Each sample was tested by X-ray diffraction for recrystallization, using known proportions of powdered calcite and aragonite as standards. As a further check, we also tested shells of *Melanoides* collected alive from the lower White Nile and from Lake Lyadu in the southern Afar Rift. Despite the unavoidably small samples used for dating, we consider the ages reliable. The lower shell bed dates to 1,710 ± 160 yr BP (SUA-1794), the upper to 1,900 ± 110 yr BP (SUA-1795).

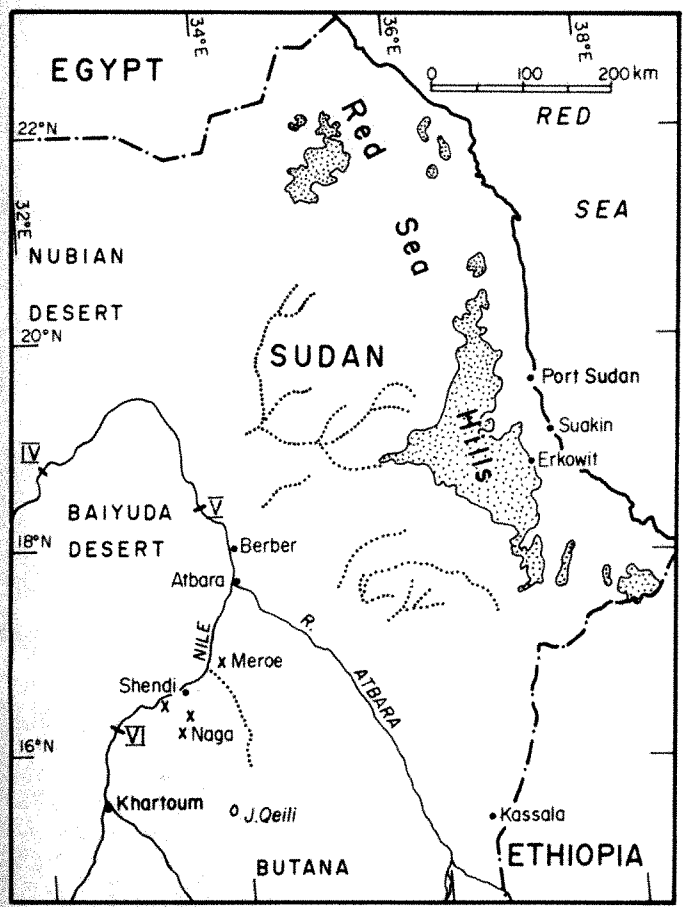


Fig. 1 Map of the northeastern Sudan showing location of Erkowit at the eastern margin of the Red Sea Hills. Crosses denote Meroitic sites (~2,000 yr BP), dotted lines are major ephemeral stream channels; land above 1,000 m is dotted; Roman numerals are Nile cataracts.

Of our ostracod assemblage, most species of *Cyprinotus* and *Darwinula* live in fresh water, but some are found in oligohaline to mesohaline water; *Canodopsis* is a freshwater form; and most *Ilyocypris* species are mud-dwellers[16].

Living non-marine charophytes inhabit fresh and brackish water[17]. They usually grow submerged in fresh standing water upon a muddy or sandy bottom, and often form extensive subaquatic meadows that may extend to depths of at least 10 m[18].

Of the molluscs, *Lymnaea* produces gelatinous eggs which dry up when removed from water. Adults can move outside water, but they are essentially aquatic as reproduction is impossible except in water. Modern habitats range from muddy or stagnant ponds and marshes to shallow, fast-flowing streams and lakes[20,21]. *Melanoides* is a wholly aquatic river or lake bottom species[21–24], but can survive in very shallow brackish water, albeit in dwarfed form, as in the eastern Afar desert. *Lentorbis junodi* lives in slow-flowing streams and marshes[24]. *Gyraulus connollyi* occurs today in stony or vegetated streams in the temperate uplands of southern Africa[24]. *Biomphalaria pfeifferi* inhabits stony and vegetated streams[24], seems intolerant of very high temperatures[25,26] and, unlike *B. sudanica*[27,28] is not found living in small seasonal pools or large swamps.

From the fossil evidence we infer that there was sufficient water on the Erkowit plateau towards 1,700–1,900 yr BP to sustain permanent stream flow in a landscape more densely vegetated than today, with flat-floored swampy meadows in the valley bottoms flanking mixed-load streams that were actively aggrading their channels with sandy or silty muds. More enduring rains during both winter and summer may have raised groundwater levels to the point where perennial base flow became possible.

Was the wetter climate at Erkowit a purely local phenomenon? We think not, as several independent lines of

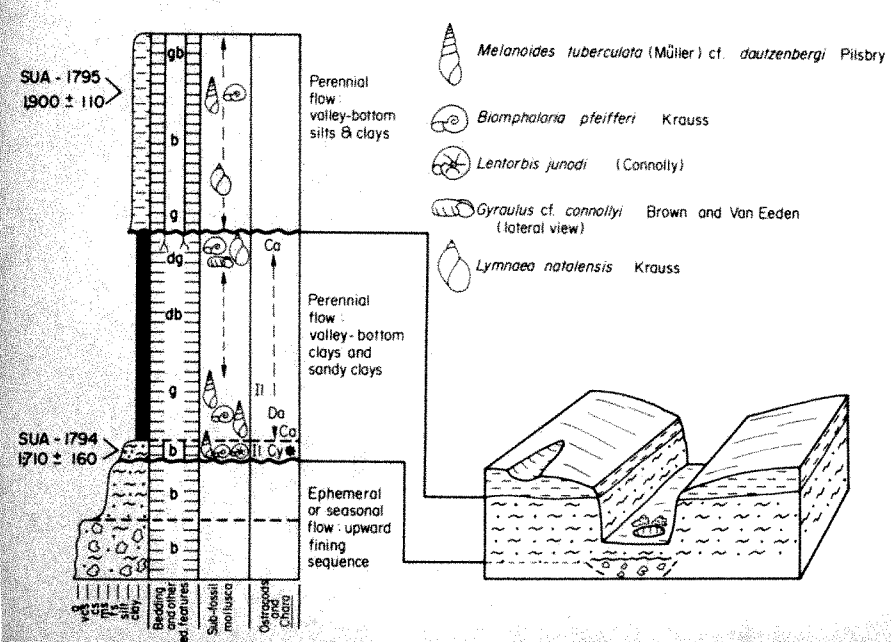


Fig. 2 Stratigraphy and local geomorphic context of the Erkowit shell-bearing clays. The ostracods are *Ilyocypris* sp. (Il), *Cyprinotus* sp. (Cy), *Canodopsis* sp. (Ca) and *Darwinula* sp. (Da). Broken arrows denote scattered distribution. The star denotes *Chara* sp. A and B. Bedding is massive (open dashes) or flat (closed dashes). Colour symbols denote brown (b), grey (g) and dark (d). The lithological symbols are clay as black, silt as dashes, sand as dots, and clay–sand mixtures as dots and wavy lines.

Table 1 Erkowit sub-fossil mollusc collections

a June 1941 Tothill collection (ref. 1)
Xerophila sp.
Cerastus abyssinica Pfv.
Planorbis herbini Byt.
Bulinus truncatus Aud.
Limnaea caillaudi Boury.
Melanoides tuberculata Mull.

b January 1973 Williams collection (this paper)
Melanoides tuberculata (Muller) cf. *dautzenbergi* Pilsbry
Lentorbis junodi (Connolly)
Biomphalaria pfeifferi Krauss
Lymnaea natalensis Krauss
Gyraulus cf. *connollyi* Brown & Van Eeden

Note that our *L. natalensis* is probably Tothill's *L. caillaudi.*

evidence point to a regionally wetter climate at about this time.

Short-lived lake transgressions are apparent at Lake Bosumtwi in Ghana soon after 2,400 BP[29], at Lake Abhe and other lakes in the Afar desert[30,31] towards 2,000–1,500 BP, and in the Ziway–Shala basin[32] of the Ethiopian Rift at ~1,600 BP. In the Chad basin several small interdune lakes are dated at 2,400 BP and 1,750 BP, at which time the Bahr el Ghazal had started to flow again[33,34]. There are also signs of reduced aridity in southern Libya between 1,930 and 1,435 BP, coeval with runoff from Tibesti[35] and with alluviation in the Hoggar[36].

In the Sudan, swamps reached to the foot of Jebel et Tomat on the lower White Nile during 1,930–1,600 BP[37]. The twin hills are now over 12 km from the closest riparian swamps[38]. The two centurions sent by Nero about AD 60 to find the sources of the White Nile reported that their way was blocked by vast swamps—the present Sudd. If the two rock outcrops that they saw within the swamp are indeed Jebel Ahmed Agha, as seems plausible, these are now 200 km beyond the northern edge of the Sudd[39]. The extensive iron smelting at Meroe (Fig. 1) presupposes abundant fuel for charcoal[40], and out in the Butana present runoff is not enough to fill the large Meroitic earth dams[39], nor to sustain much settlement[40–42]. Diodorus Siculus[39] wrote soon after 60 BC of cave-dwelling cattle herders in the Red Sea Hills who fought each other for pasture and retreated into swampy places. There are no such swamps today. In Alexandria at about this time Ptolemy recorded rare summer rains from southerly winds[39], suggesting that the Inter-Tropical Convergence Zone on occasion reached further north than it does today.

Taken together, the limnological, archaeological and archival evidence from Sudan, Egypt, Ethiopia, Chad, Libya and Ghana is entirely consistent with a regional climate somewhat wetter than today at about the time that freshwater molluscs were able to live in the now arid Red Sea Hills of eastern Sudan some 2,000 years ago.

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Nutrient depletion indicates high primary productivity in the Weddell Sea

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The Southern Ocean, and in particular the Weddell Sea, have long been considered areas of high biological productivity[1], but recent isotopic measurements of primary productivity have not confirmed this view[2,3]. Because the large zooplankton and marine mammal populations of the Southern Ocean depend ultimately on phytoplankton as the base of the food web, accurate knowledge of primary productivity is essential to our understanding of the Antarctic ecosystem. Oceanographic data collected aboard the Soviet icebreaker *Mikhail Somov* have allowed us to derive a new productivity estimate, based on the seasonal depletion of nitrate, phosphate and silicic acid in the surface layer. From these depletions and data on the elemental composition of Southern Ocean phytoplankton, we estimate average primary productivity in the Weddell Sea in the spring-time to be 220–420 mg C m^{-2} day^{-1}. Our most conservative estimate is 1.5–4 times higher than recently reported measurements of productivity in the open ocean areas of the Southern Ocean[2–5]. Our estimates are inherently averages over time and space, including the effects of brief, intense spring blooms of phytoplankton which may occur near the receding ice edge[6–8]. Studies of primary productivity based on isotope uptake experiments, particularly in the austral summer, may fail to account for the significance of such blooms.

In October and November 1981, the joint US–Soviet Weddell Polynya Expedition aboard *Mikhail Somov* penetrated several hundred kilometres into the seasonal pack ice covering the eastern Weddell Sea, roughly along the Greenwich meridian. Preliminary results, including a description of the hydrographic regime and sea ice conditions, have been reported elsewhere[9–12].

The sub-ice surface waters comprised a very homogeneous mixed layer some 60–110 m thick in which dissolved nutrient concentrations were highest at the northernmost stations, decreasing slightly in the Weddell gyre proper. Because ice conditions restricted *Somov* to a relatively small sector of the northeastern Weddell gyre, it was necessary to examine the data set in the context of data from other cruises in the same area. Comparison of the nutrient data with austral summer data from GEOSECS and *Islas Orcadas* revealed that the austral summer data exhibited evidence of marked surface layer nutrient depletions. It was also apparent that virtually unaltered remnants of winter surface waters were present at several of the austral summer stations[12]. The existence of this unaltered winter water[13] (WW) at GEOSECS station 89, near the *Somov* track, with nutrient concentrations very similar to those in the winter surface layer, led us to derive an estimate of phytoplankton productivity from the net depletion of nutrients in the overlying surface waters.

Several assumptions are implicit in this approach to the estimation of seasonal nutrient depletion: (1) the separate summer and winter data sets (that is, different cruises) are quantitatively compatible; (2) little or no vertical or lateral mixing takes place to alter nutrient concentrations in either the surface layer or the WW layer beneath it; (3) a realistic time frame can be assigned over which the depletion of nutrients occurs; (4) vertical homogeneity in the surface layer under the ice is characteristic of late winter conditions in all years.

The hydrographic data used here were collected on the GEOSECS Atlantic cruise in 1972–73, *Islas Orcadas* cruise 12 in 1977 and the *Somov* cruise in 1981. The nutrient data from the GEOSECS and *Somov* cruises were obtained using the same automated techniques[14]; on *Islas Orcadas* manual techniques were used[15]. Intercomparisons of the nutrient chemical data on deep potential temperature and density surfaces confirm the comparability of the GEOSECS and *Somov* data, but the *Islas Orcadas* data appear to be systematically lower in dissolved silicic acid and higher in phosphate. In our calculations, we have used the *Islas Orcadas* data solely to illustrate the spatial extent of the nutrient depletion. Table 1 presents the temperature, salinity and dissolved nutrient data (phosphate, nitrate and silicic acid) we used.

Table 1 and Fig. 1 illustrate the evidence for stating that minimal vertical diffusion has occurred to alter the dissolved nutrient concentrations in either the surface layer or the WW. The *Somov* data show the temperature of the entire surface mixed layer beneath the ice to be almost at the freezing point. It is underlain by warmer, more saline water and would be heated from above by insolation as the ice recedes; thus, any substantial vertical mixing across the WW layer would cause an increase in its temperature. No such increase was observed (Fig. 1). Instead, a cold lens of remnant WW exists in the Weddell Sea. This was first noted half a century ago[16] but very few chemical observations have been available until recently. For the depletion calculations discussed here, we have used only the GEOSECS station at which the WW properties were demonstrably identical to those in the wintertime mixed layer (Fig. 1).

Generally, horizontal advection and mixing could also modify nutrient concentrations in surface waters above the remnant WW layer, but in the Weddell gyre lateral transport would do little to alter the apparent depletion of the surface waters relative to the underlying WW. There is a high degree of lateral homogeneity in the Weddell Sea, particularly in the zonal direction in which the major circulation occurs[16]. Additionally, circulation within the gyre is quite weak[9,17], and vertical shear between the surface and WW is not sufficient for the surface waters observed in the austral summer to have originated very far away from the underlying WW.

Dilution of the surface layer by melt water from the receding ice pack (mean thickness = 1 m) would cause an apparent depletion of nutrients in the surface layer. Adding 1 m of melt water to a surface layer 40 m thick (that is, a dilution of 2.5%) would account for ~7% of the observed nutrient depletion. Some of the *Islas Orcadas* stations we examined had surface salinities which were diluted by as much as 5% relative to the WW, which could account for 15–20% of the apparent nutrient depletion. At the austral summer GEOSECS station used in our calculations, the surface salinity is within 1% of the salinity of the WW layer, indicating that dilution effects were negligible.

The assignment of a time frame during which the observed nutrient depletion took place is somewhat problematical. The timing of the retreat of the pack ice is highly variable from year to year, usually beginning in mid-November[18,19]. November 1972 had the greatest extent of pack ice for the decade 1966–76[19], and it is thus unlikely that the GEOSECS station location was ice free until late November or early December. (The November ice extent data are the best indication of maximum annual ice extent available for the pre-microwave satellite era[19].) The GEOSECS observations were made during January 1973; therefore, we have assigned a 60–90 day time frame to the observed nutrient depletion.

The annual cooling of the surface layer at the onset of the austral winter results in the formation of a homogeneous surface layer before ice formation[16]. The *Somov* data indicate that this layer remains homogeneous in all properties throughout the winter; therefore little or no primary production occurs below the ice to alter the nutrient concentrations. Observations of very low levels of chlorophyll *a* and extremely limited light penetration of the pack ice during the *Somov* cruise[20] support the conclusion that primary productivity under the ice pack is minimal. Thus, the homogeneity of nutrient concentrations in the surface mixed layer at the end of austral winter can be assumed to be typical.

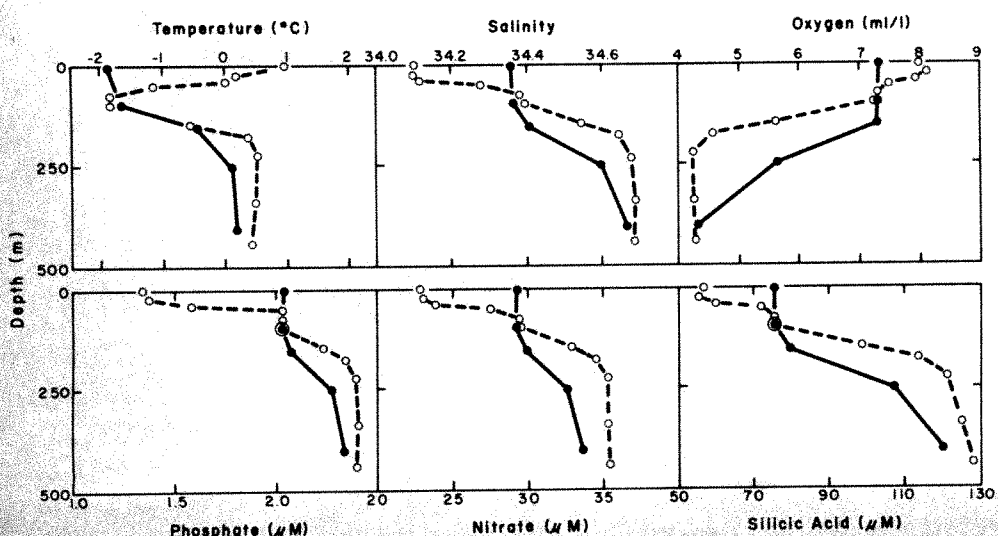


Fig. 1 Hydrographic properties at *Somov* station 35 at 59° 30′ S 0° 30′ E (solid lines) and GEOSECS station 89 at 60° 1.5′ S 0° 1.5′ E (dashed lines). The remnant winter water at the GEOSECS station is at 75–100 m.

Table 1 Winter water at GEOSECS 89 and wintertime surface water at WEPOLEX 35

Station	Pressure (db)	Potential temperature (°C)	Salinity (‰)	Silicate (μM)	Phosphate (μM)	Nitrate (μM)
GEOSECS 89	1	0.965	34.103	56.9	1.34	22.9
22 January 73	75	−1.830	34.383	75.7	2.03	29.4
60° 01′ S/0° 01′ E						
WEPOLEX 35	Entire	−1.858	34.358	75.7	2.04	29.3
12 November 81	surface					
59° 05′ S/0° 40′ E	mixed layer					

Figure 2 illustrates the spatial extent of the summer/winter contrast in dissolved silicic acid and phosphate concentrations in the eastern Weddell Sea. Because we believe the *Islas Orcadas* nutrient data to be systematically offset from the *Somov* data, we have used only the GEOSECS data to quantify the observed nutrient depletion. Taking the seasonal depletion to be the difference between the observed nutrient concentration and the WW nutrient concentration, the vertically integrated depletions at GEOSECS station 89 are 850 mmol m^{-2}, 27 mmol m^{-2} and 300 mmol m^{-2} for silicic acid, phosphate and nitrate, respectively. The average rates of depletion over the 60 and 90 day time frames assigned are shown in Table 2.

Our average nutrient depletion rates can be compared with experimentally determined rates of nitrogen and silicon assimilation by Antarctic phytoplankton. On recent cruises in the Scotia Sea[4,7], vertically integrated nitrate uptake rates were 0.74–2.41 mmol m^{-2} day^{-1} for those stations which were not in close proximity to a land mass. (An 18-h day has been used to convert from reported hourly rates.) Our most conservative estimate of the average daily nitrate depletion (Table 2) is 2.5–4 times the mean rate for these Scotia Sea stations. Similarly, for stations south of 60° S in the Pacific sector of the Southern Ocean, net production of biogenic silica in the euphotic zone was 2.5–4.5 mmol Si m^{-2} day^{-1} (ref. 21), compared with our calculated silicic acid depletion rates of 9.4–14.2 mmol m^{-2} day^{-1}. Therefore, the seasonal average depletion rates of both nitrate and silicic acid exceed by a factor of 1.5–5 the few available directly measured rates in the Southern Ocean.

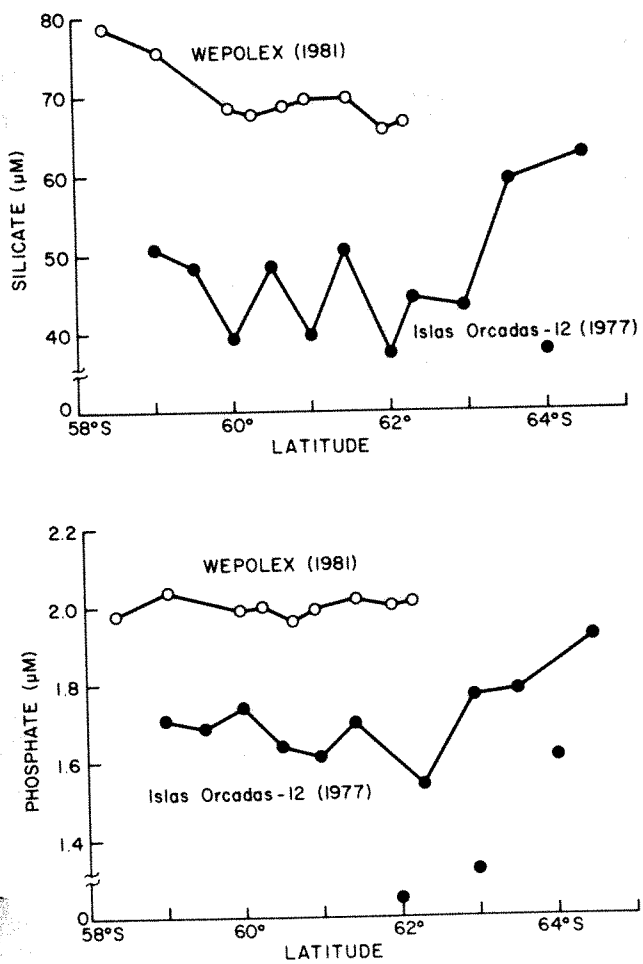


Fig. 2 Meridional distribution of dissolved silicic acid and phosphate in surface waters of the eastern Weddell Sea. *Islas Orcadas* data from summer 1977 are contrasted with *Somov* data from winter 1981 to illustrate the north–south extent of the apparent seasonal depletion.

The estimation of primary productivity from nutrient depletion requires knowledge of the stoichiometry of nutrient assimilation and carbon fixation by phytoplankton. The average composition of marine phytoplankton is often taken to be C/N/P = 106/16/1 (ref. 22), but lower C/N and C/P ratios have been reported for Southern Ocean phytoplankton[23]. Similarly, C/Si ratios for diatoms in the Southern Ocean are apparently lower than those for most marine diatoms[23]. We have used C/N/P = 62/11/1 and C/Si = 2.5 as representative of Antarctic phytoplankton[23]. Our estimates of average primary productivity are shown in Table 2. They range from ~220 mg C m^{-2} day^{-1} to 420 mg C m^{-2} day^{-1}.

Our lowest estimates of average primary productivity are based on phosphate and nitrate depletion occurring over a 90-day period. Because particulate nitrogen and phosphorus are remineralized more rapidly than is biogenic silica, they are more likely to be recycled within the euphotic zone. One aspect of this recycling is that some nitrogen is returned to the euphotic zone as ammonium by zooplankton excretion and microbial regeneration (see, for example, ref. 4). This ammonium is utilized preferentially by phytoplankton[4], so our nitrate-based estimate of primary productivity is inherently an underestimate. The estimate of primary productivity from nitrate depletion is considered to be that fraction of the total which is available for net transport up the food chain[4,7]. Available data on ammonium and nitrate uptake by Southern Ocean phytoplankton suggest that ammonium utilization supports 40–70% of the total productivity[4,7], so our estimate of nitrate utilization probably accounts for no more than half of the total. As only direct measurements of biogenic silica dissolution made in the Southern Ocean indicate that about one-third of the assimilated silicic acid dissolves in the upper 100 m[21], our silicic acid-based calculation may well be low by that amount. If our nitrate- and silicic acid-based

Table 2 Primary productivity estimated from nutrient depletion

Nutrient	Observed depletion (mmol m^{-2})	Depletion rate (mmol m^{-2} day^{-1}) 90-day interval	60-day interval	Estimated primary production (mg C m^{-2} day^{-1})
Silicic acid	850	9.5	14.2	282–426
Phosphate	27	0.30	0.45	223–335
Nitrate	300	3.4	5.1	223–335

primary productivity estimates are increased accordingly, they agree within 10%.

We believe that our calculations are conservative ones, resulting in estimates of primary productivity that are probably low, yet are 1.5–5 times higher than recent isotopic productivity measurements in the Southern Ocean[2,3,5,24]. Intense phytoplankton blooms have been reported near the edge of the receding seasonal ice pack which could easily account for much of the discrepancy between our calculated rates and those measured isotopically. Primary productivity in these blooms often exceeded 1 g C m^{-2} day^{-1} (refs 6–8, 24). As its seasonal ice cover recedes, most of the Weddell Sea surface is an ice edge zone for some period of the austral spring. A brief (10–15 day) bloom during which productivity was in excess of 1 g C m^{-2} day^{-1}, followed by a longer period of much lower productivity (closer to the ~0.1 g C m^{-2} day^{-1} values of the recent literature[2,3,5,24]), would result in seasonal average rates similar to those we derive. The contribution of such blooms is inherently included in our calculations, but may be missed by discrete measurements which are remote from the ice edge.

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Bacterial chemolithotrophy in the ocean is associated with sinking particles

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The oceanic carbon cycle has traditionally been viewed as a reversible, one step reduction–oxidation reaction ($CO_2 \rightleftharpoons CH_2O$). Principle pathways were thought to involve eukaryotic photoautotrophy and oxygen-dependent bacterial respiration, respectively. However, prokaryotic (cyanobacterial) photoautotrophy is now well documented and has even been proposed as a major carbon pathway[1–6]. In a previous study of the mesopelagic zone in the North Pacific Ocean[7], the observed downward fluxes of organic carbon, nitrogen, ATP and RNA suggested production *in situ* of new particulate organic carbon at 700–900 m. Here we present evidence that this is indeed the case and that it is mediated by bacterial chemolithotrophy. Energy for this process may be in part provided by detrital NH_4^+ derived from the downward flux of large particles.

In order to reconcile the mesopelagic zone carbon budget, chemolithotrophic production of organic matter was proposed as a possible mechanism[7]. During the Vertex III sediment trap experiment, we had an opportunity to examine the possibility of bacterial chemolithotrophic production at a station, located in the eastern tropical North Pacific Ocean approximately 330 km off Manzanillo, Mexico (15°55′ N; 107°12′ W). The Vertex III free floating sediment trap array, used for 20.6 days, consisted of 168 individual collectors at 14 depths (50–2,000 m). Details of the trap construction are given elsewhere[8]. To test the chemolithotrophy hypothesis, we used five sediment trap collectors at each of five depths (100–750 m). Measurements included: (1) concentration of ATP extracted *in situ*[9] to estimate the biomass of living microorganisms associated with sinking particles, (2) shipboard-extracted ATP concentrations[7] in unpreserved traps to estimate the net *in situ* changes in biomass during the experiment (see Table 2), (3) concentrations of NH_4^+, NO_2^- (see Table 1), (4) organic carbon and nitrogen concentrations[8], (5) rates of microbial DNA synthesis *in situ*[7], (6) microbial incorporation of $^{14}CO_2$ (see Table 2), and (7) microscopic examination of glutaraldehyde-fixed sediment trap samples. The *in situ* ^{14}C-incubation experiments and ATP measurements were performed in paired sediment traps, with and without the addition of a mixture of potential chemolithotrophic substrates (see Table 2).

Several results suggest the activity of chemolithotrophic bacteria at these depths. First, the concentration of NH_4^+ measured in the sediment trap solutions (and hence, the corresponding downward fluxes of particle-associated NH_4^+) increased between 100 and 150 m (Table 1), indicating production of NH_4^+ in association with descending particles, scavenging of NH_4^+ from the water column during descent or horizontal advection of particle-associated NH_4^+. At greater depths both concentration and flux of NH_4^+ consistently decreased. The estimated NH_4^+ fluxes are considered to be minimum values as no attempt was made to remove associated or covalently bound NH_4^+ from the sinking particles. The flux of NH_4^+ thus calculated was 383 and 149 μmol m^{-2} day^{-1} at 150 and 550 m, respectively, indicating a loss of 234 μmol NH_4^+ m^{-2} day^{-1} over this depth interval (Table 1). Since NH_4^+ was undetectable (< 0.05 μmol l^{-1}) in this portion of the water column, we suggest that the NH_4^+ is consumed either in the water column or within the sinking particles at a rate which is equivalent to the measured rates of change (mean rate of 0.59 μmol m^{-3} day^{-1} between 150–550 m). The consumption of NH_4^+ (that is, $-\Delta NH_4^+/\Delta Z$) is associated with the appearance of NO_2^- (Table 1). These data suggest the presence of active bacterial nitrification.

Nitrifying bacteria of the genera *Nitrosomonas* and *Nitrosococcus* were counted using specific immunofluorescence techniques[10,11]. Nitrifiers were detected in high concentrations in all sediment trap collections and the majority were *Nitrosomonas*-type cells. Nitrifiers were frequently observed in clusters of up to 30 cells, but single and paired cells were also common. The mean cell size varied considerably, but large cells, up to 3 μm in diameter, were common.

Table 1 Nutrient concentrations, fluxes and average rates of change over the specified depth intervals

Sediment trap depth (m)	Nutrient concentrations (μmol per sediment trap)* NH_4^+	NO_2^-	Total flux (μmol m^{-2} day^{-1}) and average rates of change (μmol m^{-3} day^{-1}) for each depth interval† Organic C flux	$(\Delta C/\Delta Z)$	Organic N flux	$(\Delta N/\Delta Z)$	NH_4^+ flux	$(\Delta NH_4^+/\Delta Z)$
100	13.8	<0.005	3,900		609		172	
				(−34)		(−7.1)		(+4.2)
150	30.8	<0.005	2,192		254		383	
				(−3.7)		(−0.42)		(−0.59)
550	12.0	2.3	717		87		149	
				(+0.55)		(+0.04)		(−1.1)
650	3.4	1.4	772		91		42	
				(+1.5)		(+0.32)		(−0.28)
750	1.1	1.1	925		123		14	

* NH_4^+ and NO_2^- concentrations were measured in 0.22 μm filtrates of the unpreserved sediment trap solutions receiving no chemolithotrophic additions (see Table 2). Samples were analysed by S. Moore within 4 h after sediment trap recovery using a shipboard Technicon Autoanalyzer system.
† Organic C and N were measured in formalin preserved sediment traps as described by Knauer *et al.*[8].

The downward flux of particle-associated nitrifiers was consistently high (1.15 to 3.52×10^8 cells m^{-2} day^{-1}) throughout the depth range of our experiment (100–750 m). Sediment traps pre-charged with chemolithotrophic substrates and incubated *in situ* produced higher cell densities, with values (relative to the control) ranging from 118% (150 m) to 263% (550 m). If we assume a maximum nitrification rate of $1.4–2\times10^{-12}$ mol NH_4^+ per cell per day (refs 12, 13) the particles collected at 550 m are potentially capable of oxidizing between 160–230 μmol NH_4^+ m^{-2} day^{-1}, a value which agrees with the observed loss of NH_4^+ at 150–550 m (Table 1).

The comparison of *in situ* versus shipboard extracted ATP can be used to ascertain the extent to which net microbial growth had occurred in the unpreserved sediment materials during the experiment[7]. It is evident (Table 2) that the two surface traps (100 and 150 m) exhibited post-incubation decreases in total ATP (that is, shipboard ATP < *in situ* ATP) indicating net microbial death. However, for that region of the water column where NH_4^+ was consumed and NO_2^- was produced (550–750 m), there was an increase in total microbial ATP during the incubation period and hence, net carbon production. The greatest increase occurred at 550 m (Table 2). At all three depths where net growth had occurred (550, 650 and 750 m), we observed an increased biomass yield following the addition of potential chemolithotrophic substrates.

The mean total $^{14}CO_2$ incorporation (Table 2) also displayed a mid-depth (550 m) maximum and, at all depths but 750 m, the incorporation rates were stimulated by the addition of inorganic substrates. However, $^{14}CO_2$ incorporation data, by themselves, cannot be extrapolated directly to microbial carbon production without information on the proportion of autotrophic versus heterotrophic processes. Heterotrophic bacteria normally assimilate 2–10% of their total carbon cell quota as CO_2 (refs 14–16), whereas certain chemolithotrophs obtain potentially 100% of their cell carbon requirements from CO_2 (refs 17, 18). By assuming the ^{14}C-incorporation data represent strictly chemolithotrophic growth, the data can be extrapolated and compared with total microbial carbon production derived from estimates of DNA synthesis (Table 3). The DNA synthesis (^3H-adenine) technique does not discriminate between the two major pathways of carbon synthesis and hence provides an estimate of total microbial carbon production. In the surface sediment traps (100 and 150 m), total microbial production greatly exceeds the ^{14}C-derived estimate, indicating that chemoheterotrophic processes predominate (Table 3). This is consistent with the observed loss of organic carbon and production of NH_4^+ associated with the large sinking particles (Table 1). However, between 550 and 750 m chemolithotrophic carbon production was detected with estimates ranging from 7% (650 m) to 90% (550 m) of the total microbial production and may therefore be a significant, if not dominant pathway for carbon and energy flow at certain depths in the mesopelagic zone.

If we assume that nitrification is the dominant pathway we can calculate a minimum amount of energy required to sustain this observed mesopelagic bacterial production. The oxidation of NH_4^+ to NO_2^- proceeds with a ΔG of −66 kcal mol^{-1}, and with typical cell yields of 0.03–0.06 mol C produced per mol N oxidized[19]. Thus at 550 m, the estimated chemolithotrophic microbial production (425 μg C m^{-2} day^{-1}) would require a flux of 787 μmol NH_4^+ m^{-2} day^{-1}, if nitrifiers were exclusively responsible, which is a value approximately three times the

Table 2 Effects of addition of exogenous reduced inorganic substrates *in situ* to sediment trap collected particles

Sediment trap depth (m)	ATP concentration, *in situ* extraction (ng per sediment trap)	Chemolithotrophic additions	ATP concentration, shipboard extraction (ng per sediment trap)	%ATP change*	^{14}C-HCO_3^- incorporation into organic matter (nCi per sediment trap)
100	1,760	No	705 ± 382	−60	11.0 ± 0.8
		Yes	1,434 ± 429	−19	16.1 ± 1.9
150	1,310	No	750 ± 6	−43	16.4 ± 0.5
		Yes	556 ± 37	−57	30.6 ± 5.3
550	110	No	748 ± 94	+580	42.7 ± 3.0
		Yes	4,004 ± 543	+3,540	48.7 ± 7.3
650	65	No	286 ± 106	+340	6.9 ± 0.2
		Yes	844 ± 271	+1,198	18.6 ± 0.7
750	220	No	662 ± 298	+201	18.3 ± 0.6
		Yes	1,198 ± 184	+445	14.5 ± 0.8

ATP associated with sinking particles was extracted *in situ* by precharging the sediment traps with a high density acid-salt solution (0.5 M H_3PO_4, 1.5 M NaCl $\rho = 1.05$ g cm^{-3}) as described by Fellows *et al.*[9]. For *in situ* incubations paired sediment traps were prepared for each depth by precharging with 2.5 l of an autoclaved high density solution consisting of 0.22 μm filtered seawater containing 33.8 g NaCl, 14.3 g $MgCl_2 \cdot 6H_2O$, 0.93 g KCl and 30 μCi $NaH^{14}Co_3$ (50 m Ci $mmol^{-1}$; New England Nuclear) per litre ($\rho = 1.065$ g cm^{-3}). At each depth, one of the paired traps received the chemolithotrophic mix consisting of NH_4Cl, $Na_2S_2O_3 \cdot 5H_2O$ and $MnCl_2$ to yield final concentrations of 400 μM NH_4^+, 400 μM $S_2O_3^{-2}$ and 100 μM Mn^{+2}. Shipboard ATP concentration refers to the value measured in the unpreserved sediment traps after recovery so as to distinguish it from the *in situ* extracted ATP. Details of the methods are presented by Karl and Knauer[7]. For ^{14}C incorporation, triplicate subsamples (100 ml each) were concentrated onto Whatman GF/F filters acidified with HCl, evaporated to dryness *in vacuo*, reconstituted with 1 ml deionized H_2O, mixed with 10 ml Aquasol (NEN) and radioassayed.
* Calculated as: [(shipboard ATP − *in situ* ATP)/*in situ* ATP] × 100%.

Table 3 *In situ* microbial production of organic matter in association with sediment trap collected particles and estimates of the role of bacterial chemolithotrophy

Sediment trap depth (m)	Chemolithotrophic additions	Minimum ^{14}C-derived microbial production* ($\mu g\ C\ m^{-2}\ day^{-1}$)	Total microbial production† ($\mu g\ C\ m^{-2}\ day^{-1}$)	Estimated % chemolithotrophic based production‡
100	No	110 ± 8	15,900 ± 3,200	< 1
	Yes	160 ± 18		
150	No	163 ± 5.4	9,900 ± 750	2
	Yes	305 ± 53		
550	No	425 ± 30	480 ± 83	90
	Yes	485 ± 66		
650	No	69 ± 2.2	1,000 ± 17	7
	Yes	185 ± 6.6		
750	No	182 ± 5.7	870 ± 31	21
	Yes	144 ± 8.4		

See Table 2 for composition of chemolithotrophic mixture.

* Calculation assumes that CO_2 represents 100% of the carbon cell quota (that is obligately chemolithotrophic) and is based upon the measured initial ^{14}C specific radioactivity of 15 μCi mmol^{-1} of total inorganic C. Values are mean ± 1 s.d.

† Total carbon production extrapolated from *in situ* measurements of rates of DNA synthesis using the conversion factors of Karl and Winn[20]. Values are mean ± 1 s.d.

‡ Calculated from data obtained without chemolithotrophic additions.

minimum *in situ* NH_4^+ consumption rate estimated from the sediment trap data (Table 1). This rate of NH_4^+ oxidation could consume potentially 1.6 mmoles $0_2\ m^-2\ day^{-1}$, if completely oxidized to NO_3^-. Therefore, chemolithotrophy (bacterial reduction of CO_2 and concomitant O_2 consumption) rather than chemoheterotrophic utilization of surface-dervied organic matter may be an important mechanism for the consumption of O_2 in the mesopelagic zone of the ocean. Although we have singled out nitrification as an important metabolic process, other pathways such as the oxidation of CH_4, H_2S, H_2 and other reduced products of anaerobic decomposition may also contribute energy for the observed mesopelagic production.

These results suggest that the oceanic carbon cycle may be more complex than previously described. The flux of energy descending from the euphotic zone may greatly exceed that nominally contained in particulate organic detritus, which has previously been considered to be the principle factor controlling the rates of mesopelagic microbiological activity and O_2 utilization. Although the flux of energy (in the form of reduced products of bacterial decomposition) required to sustain the chemolithotrophic production measured in this study is probably derived from surface primary production and thus cannot exceed it in steady-state conditions, mesopelagic 'primary' production may have a major impact on the trophic organization of localized mid-water food webs and on the production and maintenance of the intermediate depth O_2 minimum.

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Human large granular lymphocytes are potent producers of interleukin-1

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Natural killer (NK) activity[1] against tumour and virus-infected target cells is shown by a subpopulation of peripheral blood mononuclear leukocytes with the morphological features of large granular lymphocytes (LGL)[2]. The lineage of human LGL is still controversial, as they display surface markers of both T lymphocytes and myelomonocytic cells[3]. LGL have recently been reported to produce lymphokines such as interleukin-2 (IL-2)[4,5] and α- as well as γ-interferons[6], functions associated mainly with T cells. To determine whether cytokines associated with other cell lineages are also produced by LGL, we examined whether they might produce a myelomonocyte-associated cytokine such as interleukin-1 (IL-1). IL-1 is a 12–18,000 molecular weight (MW) lymphokine produced by a variety of cell types such as monocytes[7], keratinocytes[8] and a human dendritic cell line[9], which plays a crucial role in immunoregulation and inflammation[10,11]. Moreover, IL-1 has recently been reported to act synergistically with IL-2 and interferons in boosting LGL-mediated NK activity[12]. We now show that a subset of highly purified human LGL with NK activity can be stimulated to secrete a soluble factor with the biochemical and biological characteristics of human IL-1.

LGL were isolated from healthy volunteers by sequential elutriation, removal of adherent cells on a plastic surface and nylon wool columns, and centrifugation on a discontinuous Percoll density gradient[2]. Cells from the resulting fractions were tested for lysis of NK-susceptible tumour target cells and for

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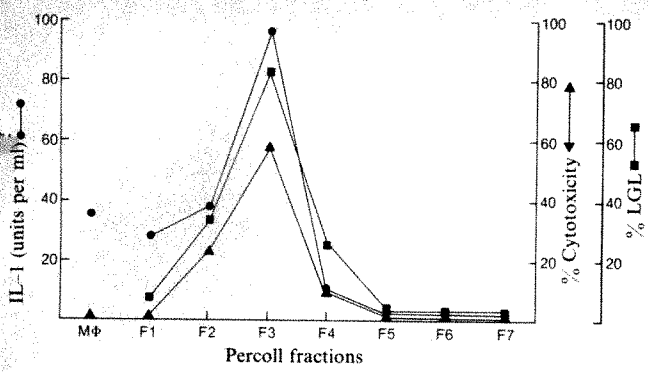
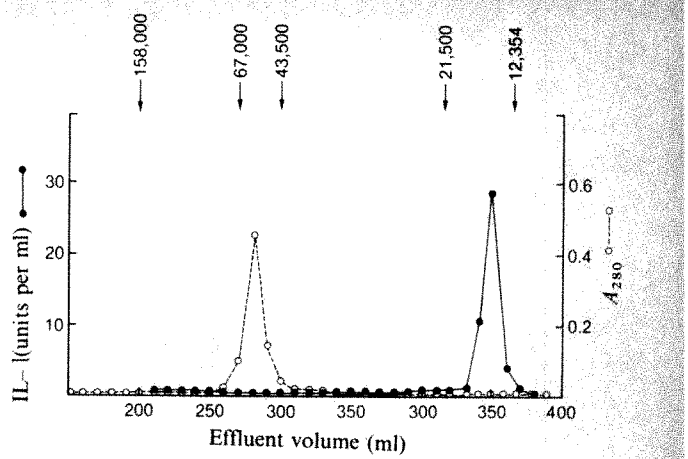


Fig. 1 IL-1 production and NK activity in fractions of peripheral blood lymphocytes separated by discontinuous Percoll density centrifugation. Peripheral blood lymphocytes were isolated and fractionated by centrifugal elutriation and lymphocyte-enriched fractions were depleted of adherent cells first by incubation on autologous serum-coated plastic flasks for 1 h at 37 °C and second, by passage over nylon wool columns. The nonadherent cells were then fractionated on a seven-step discontinuous Percoll (Pharmacia) density gradient[2]. RPMI 1640 medium + 10% fetal calf serum (FCS) and Percoll were adjusted to 285 mosmol per kg H_2O. Seven different concentrations of Percoll in medium were prepared, ranging from 40 to 57% Percoll, each varying from the next by 2.5% concentration steps. 1×10^8 peripheral blood lymphocytes were applied to each gradient and centrifuged at 550*g* for 30 min at 20 °C. The seven fractions were collected and washed. NK activity was measured in a 4 h ^{51}Cr-release assay against K562 at an effector:target ratio of 50:1 (ref. 2). For IL-1 production of 1×10^6 ml^{-1} in RPMI 1640 supplemented with 2 mM glutamine, 2 mg ml^{-1} HSA (American Red Cross) and 50 μg ml^{-1} gentamicin. Adherent monocytes were detached from the surface of plastic flask by flushing with cold PBS containing 10% FCS and resuspended at 1×10^6 ml^{-1} in the same human serum albumin-supplemented medium. Cells were then cultured for 48 h (5% CO_2, 37 °C) in the presence of 25 μg ml^{-1} LPS. Supernatants were collected, dialysed extensively against PBS (0.15 M NaCl, 0.02 M phosphate, *p*H 7.2) containing 0.01% polyethylene glycol (PEG) and 50 μg ml^{-1} gentamicin. The filtered samples (0.22 μm, Millex) were tested for IL-1 activity in a thymocyte co-stimulator assay, as described previously[30]. A single-cell suspension of C3H/HeJ (H-2^k) mouse thymocytes were cultured for 72 h at 1.5×10^6 cells per well in 96-well flat-bottomed plates in the presence of 0.5 μg ml^{-1} concanavalin A and with serial dilutions of the test samples or a standard preparation of IL-1. Results from triplicate cultures were plotted on linear regression against the standard preparation of IL-1 (set up at 100 units ml^{-1}) and expressed in units ml^{-1}. In every IL-1 assay a sample of medium with 25 μg ml^{-1} LPS was tested and was consistently negative (< 1 unit ml^{-1}). Each sample from this and the following experiments was negative for IL-2 activity when tested on the IL-2-dependent CT6 cell line[31] and was found free of contaminating IL-2. This experiment was repated four times with similar results. The percentage of LGL of each fraction of Percoll gradient was determined by morphological examination of Giemsa-stained cytopreps[2]. MΦ, macrophages.



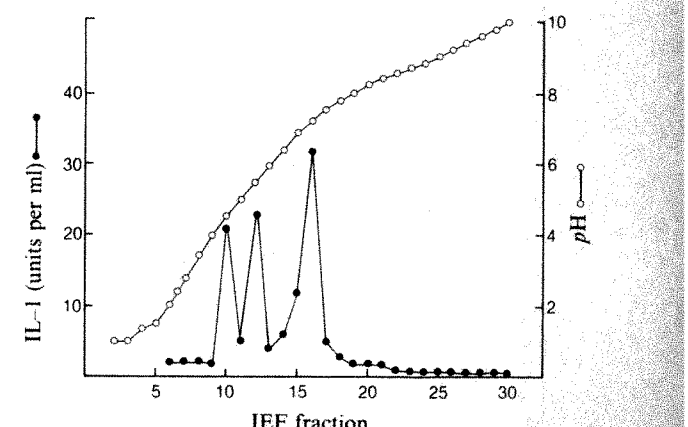


Fig. 2 Biochemical characterization of IL-1 derived from cultured LGL. 6×10^7 LGL, collected after 3 weeks' culture as described in Table 1, were washed three times with RPMI 1640 and cultured for 48 h (5% CO_2, 37 °C) in the presence of 25 μg ml^{-1} LPS at 2×10^6 cell ml^{-1}. Supernatants were collected and 47.6 g $(NH_4)_2SO_4$ per 100 ml supernatant were gradually added under gentle stirring to a final concentration of 75%. The precipitated proteins were collected by centrifugation at 5,000*g* for 10 min, resuspended in PBS and dialysed extensively against PBS (with 0.01% PEG and 50 μg ml^{-1} gentamicin). The sample was concentrated using an Amicon ultrafiltration unit with PM10 Diaflo membrane (Amicon) and dialysed against PBS as above. Aliquots of 2 ml were applied to a Sephacryl S200 (Pharmacia) packed column (2.5 × 90 cm). The column was calibrated with the following markers: aldolase (158,000 MW), bovine serum albumin (67,000 MW), ovalbumin (43,500 MW), soybean trypsin inhibitor (21,500 MW), and cytochrome *c* (12,354 MW). The sample was eluted at a flow rate of 20 ml h^{-1} with PBS (0.01% PEG, 50 μg ml^{-1} gentamicin). Aliquots (3 ml) were collected, filtered and tested for IL-1 activity as described in Fig. 1 legend. The fractions active in the IL-1 assay (Fig. 2, upper panel) were pooled, concentrated by Amicon ultrafiltration unit as described above, and dialysed extensively against 0.01% PEG. The sample was loaded onto a preparative isoelectric focusing column (LKB) in a 100 ml of 5–50% sucrose gradient with 0.01% PEG and 2% *p*H 3.5 to 10 ampholites (LKB). The *p*H gradient was formed over a period of 18 h at 1,600 V. 3-ml fractions were collected and the *p*H was determined (Fig 2, lower panel). Fractions were dialysed extensively against PBS (0.01% PEG, 50 μg ml^{-1} gentamicin), filtered and tested for IL-1 activity as described in Fig. 1 legend.

production of IL-1 upon stimulation with lipopolysaccharide *Escherichia coli* 055 B:55 (LPS) (Fig. 1). Cytochemical and cell surface analysis revealed that fraction 3 contained predominantly LGL (80–85%), enriched in cells with NK-associated markers (67% OKMI$^+$, 62% OKT10$^+$, 75% B73.1$^+$), but depleted of cells with non-NK associated markers (5% OKT3$^+$, < 1% surface Ig$^+$) and contained no detectable monocytes (< 1% esterase-positive cells)[3,13]. LPS stimulated IL-1 secretion by cells in fractions 1 and 2 which contained monocytes (30–40% and 5–10% respectively, other cells being large agranular lymphocytes) and in fractions 3 and 4 which were enriched in LGL but depleted of monocytes (< 5%) (Fig. 1). On a per cell basis, the LGL-enriched fraction 3 produced threefold higher levels of IL-1 than the monocyte-containing populations and substantially more than a purified population of adherent monocytes. Moreover addition of > 5% of autologous adherent monocytes to LGL decreased the production of IL-1 from 164 units per ml to 33 units per ml (data not shown).

LGL-enriched populations from fraction 3 were purified further to remove T cells and macrophages by treatment with the monoclonal antibodies anti-OKT3 (T-cell specific[14]) and anti-Leu-M1 (monocyte–granulocyte specific[15]) + complement (C). After this treatment, LGL (≤ 1% OKT3$^+$, < 0.3% Leu-M1$^+$) showed significantly greater LPS-induced IL-1 activity on a per cell basis than untreated LGL (Table 1).

The data in Table 1 suggested that OKT3$^+$ T cells and Leu-M1$^+$ macrophages might suppress production of IL-1 by LGL.

Table 1 Identification of the subset(s) of LGL producing IL-1

	Conditions	% Recovery $\left(\frac{\text{Final cell no.}}{\text{Initial cell no.}} \times 100\right)$ LGL	% Recovery Macrophages	IL-1 (units ml^{-1}) LGL (P)	IL-1 Macrophages	NK activity* (LU) per cell
Expt 1	C	96	95	142 (−)	72	372
	anti-Leu-M1 and anti-OKT3+C	86	7	190 (<0.05)	<1	405
	anti-Leu-M1 and anti-OKT4+C	92	ND	145 (>0.1)	ND	390
	anti-Leu-M1 and anti-OKT8+C	90	ND	309 (<0.01)	ND	239
Expt 2	LGL			102		222
	LGL OKT11$^+$			70		225
	LGL OKT11$^-$			103		166
	LGL B73.1$^+$			135		321
	LGL B73.1$^-$			15		6
	LGL			125		188
	LGL OKM1$^+$			168		481
	LGL OKM1$^-$			14		2
	LGL			156		240
	LGL DR$^+$			198		180
	LGL DR$^-$			2		215
Expt 3	LGL+C	88		150.7		160
	T cells+C	90		<1		20

Expt 1: LGL were isolated by discontinuous Percoll gradient (fraction 3) and washed three times with RPMI 1640. Aliquots were treated with optimal cytotoxic concentrations of the indicated antibody + complement (low Tox-H rabbit complement, Cedarlane) at 1:10 final dilution. Cells were washed 3× with RPMI 1640 and resuspended in human serum albumin (HSA) supplemented medium at 1×10^6 per ml. Adherent monocytes were resuspended with cold phosphate-buffered saline (PBS) containing 10% fetal calf serum (FCS) and treated with the monoclonal antibodies as indicated. Both LGL and macrophages were stimulated with 25 μg ml^{-1} LPS as described in the legend to Fig. 1. The significance level of the data, related to controls, was calculated by Duncan's multiple range analysis of variance. P values of ≥ 0.1 were considered not significant. Data are expressed as the mean of six separate experiments. **Expt 2:** LGL from fraction 3 of Percoll gradient were treated with optimal cytotoxic concentrations of anti-OKT3 and anti-Leu-M1 antibodies + C. The resulting OKT3$^-$ Leu-M1$^-$ LGL were treated with optimal concentrations of the antibodies shown (5–10 μg ml^{-1}) and then labelled with F(ab)$_2$ goat anti-mouse fluoresceinated antibody (Cappel) at 1:10 final concentration. Cells were analysed and sorted in positive and negative fractions by flow cytometry (Ortho Cytofluorograph). When reanalysed, the positive fraction contained more than 98% positive cells. The negative fraction contained less than 2% positive cells. In all the experiments, the negative cell population was further treated with cytotoxic concentrations of the same antibody + C. Cells were stimulated as described in the legend to Fig. 1. This experiment was repeated twice with similar results. **Expt 3:** LGL (fraction 3 of Percoll gradient) and T lymphocytes from Percoll fraction 7 were cultured *in vitro* for 10 generations in medium supplemented with IL-2. Lectin-free human IL-2 (ABS) was used at 10% v/v. After 3 weeks cells were collected. These cultured LGL were treated with anti-Leu-M1 antibody and C as described in Table 1 legend. The cells, free of contaminating LeuM1$^+$ or nonspecific esterase-positive cells, were then stimulated for IL-1 production as described in Fig. 1 legend. IL-1 activity was determined as reported in Fig. 1 legend. This experiment was repeated three times with similar results. ND, not determined.

* NK activity was measured against K562 cell line in a standard 4-h ^{51}Cr-release assay at different effector: target ratios. Lytic units (LU) were calculated at 30% specific lysis per 10^7 effector cells.

Table 2 Enhancing activity of LGL-derived IL-1 on T-lymphocyte response to PHA and on fibroblast proliferation

IL-1 (units per ml)	T-lymphocyte + PHA (mean c.p.m. ± s.e.m.)	CRL 1445 fibroblast (c.p.m. ± s.e.m.)
0	760 ± 88	1,270 ± 90
10	8,571 ± 720	3,819 ± 228
20	14,492 ± 650	4,959 ± 520
40	15,924 ± 790	9,863 ± 454
80	21,038 ± 1,120	10,026 ± 760

OKT3$^-$, LeuM1$^-$ LGL were stimulated with 25 μg ml^{-1} LPS at 2×10^6 cells per ml for 48 h. Supernatants were collected, dialysed extensively and tested for IL-1 activity in C3H/HeJ mouse thymocyte assay as described in the legend to Fig. 1. Monocyte-depleted T lymphocytes from fraction 7 of Percoll density gradient were cultured at 2.5×10^5 cells per well in the presence of PHA 2.5 μg ml^{-1} and the indicated concentrations of LGL-derived IL-1 for 3 days. Cultures were pulsed for the last 8 h with 1 μCi ^{3}H-thymidine (1.9 mCi mmol^{-1}). Results are expressed as mean ± s.e.m. of triplicate cultures. This experiment was repeated twice with similar results. CRL 1445 human dermal fibroblasts (American Type Culture Collection) at third passage were plated in 96-microwell plates at 1×10^4 cells per well in Dulbecco's modified Eagle's medium containing 10% heat-inactivated (56 °C, 30 min) fetal calf serum, 4 mM glutamine, 20 mM HEPES, 50 μg ml^{-1} gentamicin. CLL 1465 fibroblasts were then cultured with LGL-derived IL-1 for 3 days[23]. Cultures were pulsed for the last 16 h with 1 μCi 3H-thymidine (1.9 mCi mM^{-1}). After treatment with trypsin–EDTA solution (0.5 mg ml^{-1} trypsin, 0.2 mg ml^{-1} EDTA, 37 °C, 10 min), cells were collected using an automated device. Results are expressed as the mean ± s.e.m. of triplicate cultures. This experiment was repeated twice with similar results.

To identify the cell subset which was responsible for the observed suppression, we treated LGL with anti-Leu-M1 + C and then either with anti-OKT4 (reactive with a helper T-cell subset[16] and 4–5% of LGL[3]) or anti-OKT8 (reactive with a suppressor T-cell subset[17] and 15–20% of LGL[3]) monoclonal antibodies and C. While removal of OKT4-positive cells had no effect (see Table 1), the IL-1 production was more than doubled by treating LGL with anti-OKT8 monoclonal antibody, suggesting that a population of OKT8$^+$ LGL and OKT8$^+$ lymphocytes may regulate the IL-1 production by LGL.

To identify the subset(s) of LGL producing IL-1, OKT3$^-$, Leu-M1$^-$, LGL were sorted by fluorescence-activated cell sorter (FACS) according to their reactivity with anti-OKT11 (ref. 3), anti-B73.1 (ref. 13), anti-OKM1 (ref. 18) or AF-10 (ref. 19; a monoclonal antibody specific from DR surface molecules on macrophages and B cells) + C. The results (Table 1) showed that the IL-1 production in response to LPS was predominantly attributable to a B73.1$^+$, OKM1$^+$, DR$^+$ subset of LGL, while the B73.1$^-$, OKM1$^-$, DR$^-$ cell subsets produced only a small level of IL-1 activity. The results also showed that both OKT11$^+$ and OKT11$^-$ subsets of LGL produced comparable levels of IL-1 activity.

Human LGL maintaining NK activity can be cultured for several weeks in lectin-depleted IL-2 containing medium[20]. LGL-enriched fraction 3 and T lymphocyte-enriched fraction 7 from discontinuous Percoll density gradients were cultured in IL-2-containing media for about 10 generations. These cultured LGL and T cells were stimulated with LPS, and IL-1 production was determined during a 2-day culture period. LGL cultured for up to 3 weeks maintained their NK activity as previously reported[20], as well as their capacity to produce consistently

significant levels of IL-1 activity in the absence of any contaminating monocytes (Table 1). In contrast, cultured T cells did not produce any detectable IL-1 activity and showed no significant NK activity.

In the LGL subset(s) reactive with anti-B73.1 or anti-OKM1 monoclonal antibodies, NK activity correlated with the levels of IL-1 production. The relationship of NK activity and IL-1 production of cells separated on the basis of reactivity with anti-OKT11 monoclonal antibody was evaluated. Both the $OKT11^+$ and $OKT11^-$ subsets of LGL secreted IL-1 and showed NK activity. In the case of AF-10, however, NK activity was nearly equally distributed in both the DR^- and DR^+ subsets of LGL as reported elsewhere[3], whereas the IL-1 production was limited to the DR^+ subset.

The IL-1 activity of supernatants from cultured LGL ($\sim 6 \times 10^7$) stimulated with LPS for 48 h was characterized biochemically by sequential $(NH_4)_2SO_4$ precipitation, Sephacryl S200 gel filtration and preparative isoelectrofocusing (IEF). IL-1 activity derived from cultured LGL eluted from S200 gel column in the range of 15,000 molecular weight (MW) and was heterogeneous on IEF with a major peak at a *p*I 6.8 and two minor peaks at *p*I 5.5 and 4.5 (Fig. 2). The IL-1 activity in supernatants derived from fresh LGL had almost identical characteristics (data not shown). These data are consistent with those reported for monocyte-derived human IL-1 (ref. 21).

Monocyte-derived human IL-1 has been reported to enhance the T-lymphocyte response to polyclonal stimulants such as phytohaemagglutinin A (PHA) in the apparent absence of macrophage accessory cells[22] and to stimulate human fibroblast proliferation[23]. Similarly, LGL-derived human IL-1 could replace the requirement of T-lymphocyte response to PHA as well as to stimulate the proliferation of CRL 1445 human fibroblasts (see Table 2).

IL-1 promotes the generation of cytotoxic effector cells[24], has a crucial role in T- as well as B-lymphocyte responses to antigens[25,26], stimulates fibroblast proliferation[23] and collagenase production[27], and induces fever[28] and production of acute phase proteins[29]. The evidence that LGL produce considerable IL-1 activity suggests that some immunological function(s) in addition to NK lytic activity may be exerted by a subset(s) of LGL. Indeed, we have obtained data showing that the IL-1-producing subset(s) of LGL, $OKM1^+$, DR^+, can also exert an effective accessory cell function for T-lymphocyte activation (data not shown).

In conclusion, our results show that LGL with NK activity can produce IL-1. They show further that IL-1 was secreted mainly by LGL bearing the $OKM1^+$ or $B73.1^+$ phenotypes, which are those highly associated with NK activity. Furthermore, preliminary studies showed that human LGL-derived clones, with $B73.1^+$ $OKM1^+$ phenotype had NK activity and also secreted IL-1, suggesting that these two functions may be carried out by the same cell. It has been reported that another subset(s) of LGL is capable of producing IL-2[5]. Thus, at least two functionally and phenotypically distinct subsets of LGL have been identified; our data also raise the possibility that a third subset, of $OKT8^+$ LGL, may participate in the negative regulation of IL-1 production by LGL.

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Mouse Ia antigens are receptors for lactate dehydrogenase virus

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Infection of mice with lactate dehydrogenase virus (LDV) leads to elevation of plasma lactate dehydrogenase, lifelong viraemia and perturbations of cell-mediated and humoral immune responses. The virus replicates exclusively in a restricted set of macrophages[1,2], but the basis for restricted cell susceptibility is unknown. By immunofluorescence techniques we have found that the per cent infected was the same as the per cent expressing antigens encoded by the *I* region of the major histocompatibility complex (Ia). Infection of CBA strain $I\text{-}A^+$ peritoneal macrophages was blocked when cells were treated simultaneously with monoclonal antibody to I-A and I-E, but not with either antibody separately. LDV infectivity was inactivated when virus was treated with purified rat glycoprotein homologous to mouse I-A and I-E antigens. These results indicate that the receptors for LDV are I-A and I-E antigens. Selective infection of Ia-positive macrophages may have an important effect on the immunological capability of infected mice.

LDV (a strain received from Dr K. E. K. Rowson), in the form of serum taken 24 h after intraperitoneal (i.p.) infection of mice, was added to resident peritoneal macrophages of CD1 mice. Infected (antigen-containing) cells were scored by fluorescent antibody staining 10 h later. As the multiplicity of infection increased to 50 ID_{50}s (50% infectious dose) per cell, 4–10% cells became infected but this did not increase at 10,000 ID_{50}s per cell. Stueckmann *et al.*[3,4] have obtained similar results and also noted that it became progressively more difficult to infect peritoneal macrophages when they were maintained *in vitro* for longer than 24 h. Peritoneal macrophages are known to become Ia-negative after 1–2 days in culture[5].

Macrophages were obtained from various sites in CD1 mice and the percentage of cells supporting LDV replication and the percentage that were Ia-positive were determined (Table 1). Macrophages from all sites tested showed a close correlation between Ia positivity and susceptibility to LDV.

The expression of Ia antigens on macrophages can be regulated by prostaglandins of the E series[6]. Peritoneal macrophages

Table 1 Susceptibility to LDV infection and Ia positivity in macrophages from CD1 mice

Macrophage source	Ia-positive (%)	LDV-positive (%)
Peritoneal	5.5	4.9
Spleen	17.6	14.3
Liver	24.5	24.8
Thymus	65.2	61.0
Peripheral blood	6.7	5.9
Peritoneal macrophages from mice injected with indomethacin	25.8	23.5
Peritoneal macrophages from mice injected intraperitoneally with lymphokines	16.9	16.5

Spleen and thymus macrophages were obtained by mincing with forceps and lysing red cells with 0.83% NH_4Cl buffer. Liver macrophages were obtained by digesting with 0.05% collagenase. Peripheral blood monocytes were separated by Ficoll–Hypaque. Cells were incubated in minimal essential medium containing 10% fetal calf serum in 'ring' cultures at 37 °C with 5% CO_2 in air. After 3 h, cultures were washed vigorously to remove non-adherent cells, fixed for 10 min at 20 °C with 1% paraformaldehyde and the % Ia^+ cells determined by indirect fluorescent antibody staining with monoclonal antibody to I-A (OX6 used at 10 μg ml^{-1}). Other cells were infected with LDV at a multiplicity of infection of 10^4 ID_{50}s per cell. After 10–12 h, cultures were washed, dried, acetone-fixed and stained by the indirect fluorescent antibody technique for LDV antigens using mouse hyperimmune serum. The % of LDV-infected or Ia^+ cells was determined from examination of 20–50 fields, containing 500–1,000 cells, on each of 3–6 ring cultures. Counts on each ring culture varied by less than 20%. Each type of macrophage was obtained from a separate mouse or pool of mice and in repeat experiments the per cent Ia^+ and LDV-positive cells varied by up to twofold. In a given macrophage preparation, however, the % Ia^+ and LDV^+ were very similar.

were taken from mice that had received 50 μg indomethacin i.p. every 12 h for 72 h, and infected with LDV. The proportion of Ia-positive (Ia^+) cells was 25.8% and an equivalent number of cells were infected (Table 1). The percentage of Ia^+ cells was then increased by *in vivo* treatment with 0.1 ml of a 50% dilution of a 24-h supernatant from concanavalin A (Con A)-treated mouse spleen cell cultures; this was injected i.p. into mice every 12 h for 72 h and peritoneal cells were taken 12 h after the last injection. 16.7% of the cells were Ia^+ and 16.5% of the cells were infected. On the other hand, when peritoneal macrophages were maintained for 24 h *in vitro* before infection only 1–3% of the cells were Ia^+ and infectibility decreased likewise. A double labelling experiment was carried out in which peritoneal macrophages from normal CD1 mice were infected with LDV and stained for Ia and LDV antigens with rhodamine- and fluorescein-conjugated antibody after 7 h. The percentage of Ia^+ cells (11.0% at the initiation of infection) had decreased to 5.2% as a result of maintenance *in vitro* for 10 h; 6.1% of LDV-positive cells were now Ia-negative, but 84.6% of the remaining Ia-positive cells were LDV-positive.

The above observations show that the presence of Ia antigens in macrophages is correlated with susceptibility to LDV. This was investigated more directly by blocking infection with antibody to Ia antigens. Peritoneal macrophages from various strains of mice were treated with monoclonal antibodies that react with mouse I-A (OX6 and OX3; refs 7, 8) and with mouse I-E (H10-81.10; ref. 9), before infection with LDV. When antibody to I-A or I-E was used alone there was no influence on the virus-susceptibility of macrophages from CBA mice but there was a reduction in infection when monoclonal antibody OX6 (reacting with I-A on CBA macrophages) and antibody to I-E were added at the same time (Table 2). The monoclonal antibody OX6 does not react with BALB/c I-A, and in BALB/c macrophages the addition of monoclonal antibody to I-E fails to block LDV infectivity. I-E antigens are not expressed in *H-2b*, *H-2f* and *H-2q* mice[10] and in macrophages from C57BL/6 (*H-2b*) mice, monoclonal antibody to I-A (OX3) alone blocked the infectivity of LDV. An experiment with macrophages from I-E-negative congeneic mouse strains (Table 3), showed that the monoclonal antibody to I-A did not block LDV infection unless it reacted with macrophages[7]. As would be expected (data not shown), blocking was found to be less effective when a higher multiplicity of infection (500 ID_{50}s per cell) was used. Blocking of LDV infection was never complete, perhaps because large amounts of antibody are needed to block 100% of virus receptors on cells. Monoclonal antibody to mouse macrophages, or to mouse macrophage Fc receptors, failed to influence the LDV-susceptibility of peritoneal macrophages.

The results above were obtained by fluorescent antibody staining. On one occasion cultures were treated with actinomycin D, pulsed with tritiated uridine, and infected cells counted after

Table 2 Effect of monoclonal anti-Ia antibodies on the susceptibility of peritoneal macrophages to infection with LDV

Antibody dilution	C57BL/6 ($H-2^b$)	% Inhibition of infection: CBA ($H-2^k$) Anti-I-E dilutions			BALB/c ($H-2^d$) Anti-I-E dilutions		
		—	1:128	1:256	1:512	—	1:128	1:256	1:512
OX3	+	−							
1:128	68.6 ± 6.1*	4.5 ± 1.3	−2.2 ± 4.2						
1:256	56.9 ± 6.7†	6.7 ± 2.8		2.2 ± 8.8					
1:512	47.1 ± 4.0†	−6.6 ± 4.5			11.1 ± 6.0				
OX6	−	+				−			
1:128	−14.7 ± 5.3	4.4 ± 2.9	73.3 ± 1.8*			−8.8 ± 11.1	−17.0 ± 7.5		
1:256	−12.1 ± 5.6	−2.2 ± 2.1		53.3 ± 3.3*		−21.4 ± 5.2		−13.0 ± 5.1	
1:512		−22.2 ± 5.1			40.0 ± 3.5*				−12.1 ± 2.4
Anti-I-E	−	+				+			
1:128	11.7 ± 5.4	13.3 ± 3.3				−24.3 ± 10.5			
1:256	2.0 ± 3.9	−17.7 ± 6.4				−12.2 ± 12.7			
1:512	−2.0 ± 12.2	−22.2 ± 6.2							
Anti-Fc receptor	+								
1:1	−3.9 ± 9.2								
Anti-macrophage	+								
1:50	13.1 ± 2.3								

Resident peritoneal macrophages from C57BL/6, CBA and BALB/c mice were cultured in rings for 4–5 h after cell seeding. After removing non-adherent cells, macrophages were pretreated with monoclonal antibody to I-A (OX3, OX6), I-E (H10-81-10) or with a combination of anti-I-A and anti-I-E antibodies for 15 min at 20 °C. As controls, cells were treated in a similar way with rat monoclonal anti-mouse Fc receptor antibody (2-4G2), used as a culture supernatant[17,18] or anti-mouse macrophage antibody (F4/80) used as a concentrated culture supernatant[19]. After three washes with phosphate-buffered saline, the cells were infected with LDV at multiplicity of infection (MOI) = 50 at 37 °C for 40 min. The cultures were re-fed with minimal essential medium supplemented with 10% fetal calf serum and incubated for 8–10 h at 37 °C in 5% CO_2. The slides were then processed as described in Table 1, and LDV antigen-positive cells were counted in 1,000–2,000 cells on each of two rings. Per cent inhibition of infection was calculated as the difference between control and test divided by the control × 100. Each value represents the mean ± 1 s.e. from two experiments. *P* values were calculated by Student's *t*-test. Reactivity pattern of cells from different mouse strains with each monoclonal antibody was checked by indirect immunofluorescence. Anti-I-E antibody was used simultaneously with OX3 or OX6.
* $P < 0.001$; † $P < 0.005$.

Table 3 Inhibition by anti-I-A antibody of LDV infection of macrophages from I-E-negative mouse strains

OX6 dilution	% Inhibition of infection B10A (4R) ($H\text{-}2^{k/b}$)	B10.M ($H\text{-}2^{f}$)	B10.G ($H\text{-}2^{q}$)	C57BL/10 ($H\text{-}2^{b}$)
1:128	75.0±2.1*	70.6±5.1‡	−6.3±1.2	−1.0±5.2
1:256	63.6±2.1*	55.9±11.2§	9.5±3.0	−1.0±7.8
1:512	61.4±3.5†	8.8±1.7	−9.9±1.4	5.6±5.0
Reactivity of cells with OX6	+	+	−	−

Peritoneal macrophages from I-E-negative mouse strains were treated with monoclonal antibody to I-A (OX6), infected with LDV and the results are expressed as % inhibition of infection as shown in Table 2. The reactivity pattern of cells with this antibody is shown as described elsewhere[7] and was confirmed by immunofluorescence staining. Each value is the mean ± 1 s.e. of four ring cultures.

* $P<0.001$; † $P<0.005$; ‡ $P<0.01$; § $P<0.025$.

Table 4 Inhibition of LDV infection by purified Ia antigens

Protein concentration ($\mu g\ ml^{-1}$)	% Inhibition of infection I-A antigen	I-E antigen	Thy 1.2 antigen
100	97.0±2.8*	87.2±2.9*	1.3±8.9
10	58.3±4.5†	66.0±13.3‡	−2.3±9.6
1	33.3±11.1§	31.9±8.0§	
0.1	16.6±13.0	2.1±10.6	

Serial 10-fold dilutions of purified rat Ia antigens (homologous to mouse I-A and I-E antigen) and mouse Thy 1.2 antigen were incubated with LDV ($1\times10^7\ ID_{50}s\ ml^{-1}$) for 1 h at 4 °C, then added to C57BL/6 mouse peritoneal macrophages at a calculated MOI of 20 ID_{50}s per cell. LDV antigen-positive cells were counted in 1,000–2,000 cells for each of four ring cultures 10 h later. The data are presented as described in Table 2. The antigens were prepared by 2% deoxycholate extraction of spleen cells, affinity column purification with monoclonal antibodies, elution, and removal of deoxycholate by ethanol precipitation.

* $P<0.001$; † $P<0.005$; ‡ $P<0.01$; § $P<0.05$.

autoradiography[2]. Blocking of infection of CBA macrophages by anti-I-A plus anti-I-E antibody, but not by either antibody alone, was confirmed (data not shown).

In double-labelling experiments with fluorescein- and rhodamine-labelled antibody using rabbit antiserum to I-E and mouse monoclonal antibody to I-A, it was noted that I-A and I-E antigens were present on the same peritoneal macrophages. Rabbit anti-rat I-E antiserum plus complement was therefore used to lyse Ia-positive macrophages. With serum dilutions up to 1:100 there was a 10% reduction in cell numbers and a 90% reduction in susceptibility to LDV (data not shown).

Finally, a non-immunological method was used to show that Ia antigens are receptors for LDV. LDV was pre-treated with purified rat Ia antigens, either E or A type glycoprotein, which are homologous to mouse I-A and I-E antigens[8], then infectivity was assayed in C57BL/6 (I-E-negative) peritoneal macrophages (Table 4). At a calculated multiplicity of 20 ID_{50}s per cell, a dose-dependent significant inhibition of infection of macrophages was seen with dilutions of either antigen down to 1 $\mu g\ ml^{-1}$. Murine cytomegalovirus is another virus that infects only a minority of peritoneal macrophages[11] but this was not influenced by treating macrophages with antibody to Ia antigens, nor by treating the virus with Ia antigens (data not shown).

If Ia antigens function as receptors for LDV, the susceptibility of a subset of macrophages is accounted for. Stueckemann *et al.*[4] suggested that infected macrophages are eliminated, lifelong infection in mice being maintained by continuous production of fresh susceptible macrophages. This is explained if Ia-positive (virus-susceptible) macrophages are constantly generated. We have observed that the proportion of Ia-positive peritoneal macrophages is lower (1–3%) in persistently infected mice, suggesting selective loss of these cells.

Selective infection of Ia-positive, antigen-presenting cells might be expected to lead to perturbation of immune responses. If the cells that present viral antigen are at the same time the source of antigens, and are possibly damaged or destroyed, this could result in impaired immune responses to the infecting virus. Isakov *et al.*[12] reported impaired presentation of antigens by LDV-infected macrophages. Others have described enhanced antibody responses early after infection and depressed responses at a later stage, but the mechanisms are unclear[13]. Defects in cell-mediated responses have been described[14], and there is so far no evidence that mice develop cell-mediated immune responses to LDV.

It might have been expected that ubiquitous antigens encoded by the major histocompatibility complex would, on occasion, be used as receptors by an infecting virus but so far there has been little evidence for this. Mouse H-2 antigens can function as receptors for Semliki Forest virus[15] but these are not the only receptors because H-2-negative mouse cells can be infected with this virus[16]. Perhaps LDV can, on occasion, use non-Ia receptors.

The possibility that other Ia-positive cells, such as Langerhan's cells, B cells, dendritic cells and endothelial cells are also infected by LDV is being investigated.

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A synthetic peptide that is a bombesin receptor antagonist

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The tetradecapeptide bombesin was originally isolated from frog skin[1]. Bombesin-like peptides have since been detected in mammalian gastrointestinal tract[2,3], brain[3,4] and lung[5,6]. These peptides have potent pharmacological effects on the central nervous system[7]; they cause contraction of intestinal, uterine and urinary tract smooth muscle[8]; and stimulate the release of other peptides including gastrin[2,3,9], cholecystokinin[9], motilin[9], pancreatic polypeptide[9], neurotensin[9], insulin[9,10], enteroglucagon[9,10], prolactin[11,12] and growth hormone[11,12]. Specific plasma membrane receptors for bombesin have been

demonstrated on pancreatic acinar cells[13], brain membranes[14] and pituitary cells[15]. Studies defining the physiological importance of bombesin have been impeded by the lack of a bombesin receptor antagonist. Here we describe experiments which demonstrate that a peptide originally described as a substance P receptor antagonist[16], [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]substance P, is also a bombesin receptor antagonist. This peptide competitively inhibits the ability of bombesin to stimulate enzyme secretion from dispersed pancreatic acini, and also inhibits the action of other peptides that interact with the bombesin receptor.

A recent study of the ability of analogues of substance P (SP) to act as specific, competitive substance P receptor antagonists, showed that high concentrations of one analogue, [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP, also inhibited bombesin-stimulated amylase release[17]. Because there are no known competitive inhibitors of bombesin, we investigated this inhibition in more detail, and now report that [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP is a bombesin receptor antagonist.

Dispersed acini from guinea pig pancreas were prepared as described previously[18,19]. Previous studies have shown that these acini possess specific, high-affinity plasma membrane receptors for bombesin[13], substance P[20], muscarinic cholinergic agents[21], cholecystokinin[22], vasoactive intestinal peptide (VIP)[21,23] and secretin[21,23,24]. The bombesin receptor interacts only with bombesin and structurally related peptides[13,21] (litorin, ranatensin, alytesin and gastrin-releasing peptide) and occupation of the bombesin receptor by any of these peptides causes mobilization of cellular calcium and stimulation of enzyme secretion[13,25,26].

Dispersed acini were suspended in a standard incubation solution containing 24.5 mM HEPES (*p*H 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM NaH_2PO_4, 5 mM sodium pyruvate, 5 mM sodium fumarate, 5 mM sodium glutamate, 2 mM glutamine, 16.5 mM glucose, 0.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5 mM theophylline, 1% (w/v) albumin, 0.01% (w/v) trypsin inhibitor, 1% (v/v) amino acid mixture and 1% (v/v) essential vitamin mixture. The incubation solution was equilibrated with 100% O_2 and all incubations were performed with 100% O_2 as the gas phase.

Bombesin caused a 10-fold stimulation of amylase release (Fig. 1*a*). Detectable stimulation occurred with 0.03 nM bombesin, half-maximal stimulation with 0.16 nM bombesin and maximal stimulation with 1 nM bombesin. [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP in concentrations up to 100 μM did not stimulate amylase release but caused a parallel rightward shift in the dose–response curve for bombesin-stimulated amylase release (Fig. 1*a*). With increasing concentrations of [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP, the magnitude of the rightward shift was proportional to the concentration of the peptide (Fig. 1*a*). When the data from Fig. 1*a* were plotted in the form of Schild[27], that is, log(dose ratio − 1) versus log antagonist concentration, the values could be described by a single regression line having a slope (and 95% confidence limits) of 1.00 (0.84–1.15) (Fig. 1*b*). These results demonstrate that in addition to being a competitive antagonist of the substance P receptor on these acinar cells[17], [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP is also a competitive antagonist of the bombesin receptor. The apparent affinity (and 95% confidence limits) of [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP for the bombesin receptor calculated for bombesin-stimulated amylase release was 2.9 (0.7–12) μM. Previously, studies have demonstrated that bombesin and litorin have affinities for the bombesin receptor of 2 nM and 40 nM, respectively[13]; therefore, the apparent affinity of [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP for the bombesin receptor is 1,500-fold lower than that for bombesin and 70-fold lower than that for litorin. The affinity of [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP for the substance P receptor on pancreatic acini has been shown to be 0.65 μM[17]; thus, the substance P analogue has four times greater affinity for the substance P receptor than for the bombesin receptor.

[D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP inhibited the stimulation of amylase release by peptides that interact with the bombesin

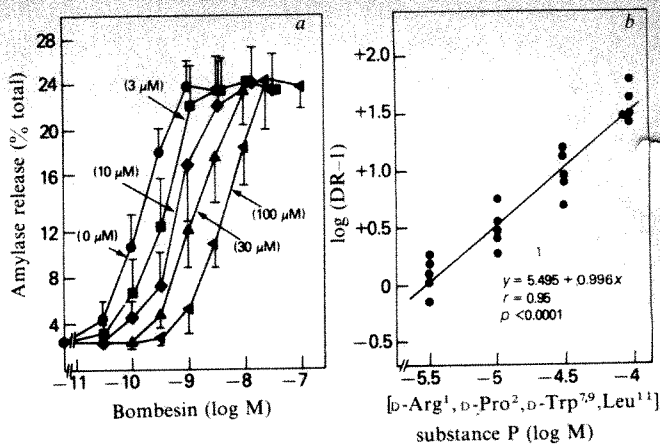


Fig. 1 Effect of various concentrations (shown in parentheses) of [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP on bombesin-stimulated amylase release. *a*, Dispersed acini from guinea pig pancreas were incubated with 0, 3, 10, 30 or 100 μM [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP with the indicated concentration of bombesin. Amylase release was measured during a 30-min incubation at 37 °C as described previously[30] and is expressed as the percentage of the amylase in the acini at the beginning of the incubation, that is, the percentage of the total. In each experiment each value was measured in duplicate and results are the means from five separate experiments. Vertical bars represent 1 s.d. Data from the experiments shown in *a* were plotted in the form of Schild[27] (*b*). The best fit to the line was calculated by least-squares analysis. DR, the dose-ratio, which is the ratio of the dose of bombesin required to give half-maximal stimulation in the presence of a given concentration of [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP to the dose required to give half-maximal stimulation in the absence of [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP.

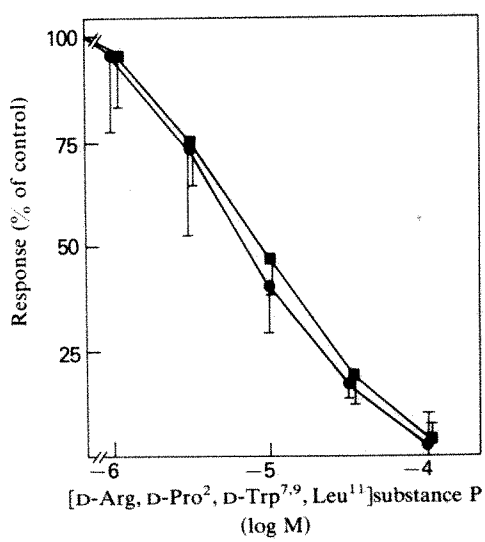


Fig. 2 Effect of [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP on bombesin-stimulated amylase release (●) and on binding of ^{125}I-[Tyr[4]]bombesin (■). To measure amylase release, dispersed pancreatic acini were incubated with or without 0.3 nM bombesin plus the indicated concentrations of [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP. Values for amylase release were calculated as the percentage of the increase caused by bombesin alone. To measure binding of ^{125}I-[Tyr[4]]bombesin, acini were incubated with 0.3 nM ^{125}I-[Tyr[4]]bombesin plus the indicated concentration of [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP. Saturably bound ^{125}I-[Tyr[4]]bombesin was determined after incubation at 37 °C for 30 min as described previously[12]. Values for ^{125}I-[Tyr[4]]bombesin bound are calculated as the percentage of radioactivity that was saturably bound in the absence of added [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP. In each experiment each value was measured in duplicate and the results are the means of six separate experiments. Vertical bars represent 1 s.d.

receptor (bombesin, ranatensin, gastrin-releasing peptide, litorin and alytesin)[8,13], or by peptides that interact with the substance P receptor (substance P)[20] (Table 1). In contrast, [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP had no effect on stimulation caused by agents that interact with the cholecystokinin receptor (CCK-8), the cholinergic receptor (carbachol) and the vasoactive intestinal peptide receptor (VIP, secretin), or by the secretagogues 8-bromo-cyclic AMP and A23187 (Table 1).

To investigate directly the ability of [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP to interact with bombesin receptors on pancreatic acini, we measured its ability to inhibit binding of ^{125}I-[Tyr^4]bombesin and its ability to inhibit bombesin-stimulated amylase release in identical experimental conditions. For each function, the dose–inhibition curves were superimposable (Fig. 2). For both inhibition of binding of ^{125}I-[Tyr^4]bombesin and inhibition of bombesin-stimulated amylase release, [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP caused detectable inhibition at concentrations greater than 1 μM, half-maximal inhibition at 7.2 μM, and complete inhibition at 100 μM. These results demonstrate directly that [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP interacts with the bombesin receptor and that its ability to inhibit bombesin-stimulated amylase release can be accounted for entirely by its ability to interact with the bombesin receptor.

Table 1 Effect of [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP on the stimulation of amylase release by various pancreatic secretagogues

Secretagogue	Amylase release (% total)	
	Secretagogue alone	[D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP (30 μM)
None	2.6 ± 8.1	2.7 ± 1.2
Bombesin (0.3 nM)	17.5 ± 5.0	3.8 ± 1.2*
Ranatensin (0.3 nM)	20.6 ± 4.3	6.0 ± 2.4*
Gastrin-releasing peptide (1 nM)	18.8 ± 3.0	6.0 ± 2.4*
Litorin (1 nM)	24.6 ± 5.4	5.0 ± 1.9*
Alytesin (1 nM)	17.3 ± 5.2	4.2 ± 2.8*
CCK-8 (0.1 nM)	31.7 ± 4.7	30.0 ± 3.4
Carbachol (10 μM)	31.1 ± 5.0	29.2 ± 2.3
A23187 (5 μM)	14.0 ± 2.5	14.8 ± 1.2
VIP (3 nM)	26.8 ± 2.0	27.2 ± 2.4
Secretin (0.1 μM)	26.0 ± 3.5	26.3 ± 2.7
8-bromo-cyclic AMP (1 mM)	24.0 ± 4.0	25.6 ± 1.2
Substance P (3 nM)	7.4 ± 1.4	2.4 ± 0.6*

Dispersed acini from guinea pig pancreas were incubated for 30 min at 37 °C with various secretagogues alone or with [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP. In each experiment each value was determined in duplicate and results are the means ± 1 s.d. from four separate experiments. CCK-8, the COOH-terminal octapeptide of cholecystokinin.

* Significantly different ($P < 0.01$) from the value obtained with the secretagogue alone by Student's paired t-test.

The apparent affinity of [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP for the bombesin receptor is much greater than that of substance P, because the latter, at concentrations as high as 30 μM, does not interact with bombesin receptors on pancreatic acini (data not shown). In contrast, the affinity of [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP for the substance P receptor on pancreatic acini is 2,500-fold lower than that of substance P[17]. Thus, modifying substance P by substitutions in positions 1, 2, 7, 9 and 11 increases the affinity of the peptide for bombesin receptors but decreases the affinity of the peptide for substance P receptors.

Several different analogues of bombesin have been tested and found not to inhibit the action of bombesin[28,29]. Our present finding that an analogue of substance P can function as a bombesin receptor antagonist raises the possibility that appropriate modifications of the bombesin molecule may give rise to a peptide that will function as a bombesin receptor antagonist without also interacting with the substance P receptor.

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The second messenger linking receptor activation to internal Ca release in liver

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The increase in cytosolic [Ca^{2+}] induced by Ca-mobilizing hormones in liver is mainly due to release of Ca from intracellular stores[1–7]. For Ca to be released from internal sites a messenger must be formed at the plasma membrane which diffuses into the cytosol to signal Ca release from the intracellular organelles[2,3,7,8]. One of the first actions of these hormones is to cause breakdown of the polyphosphoinositides to form soluble inositol phosphates[9–11]. Some evidence for the idea that these substances could be the second messenger[10] has been obtained in pancreatic acinar cells[12]. Here we have found that hormone activation of hepatocytes causes rapid breakdown of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] to form inositol trisphosphate ($InsP_3$). When applied to permeabilized hepatocytes, $InsP_3$ releases Ca from non-mitochondrial ATP-dependent pools. This suggests that $InsP_3$ could be the messenger linking Ca-mobilizing receptor activation to intracellular Ca release in liver.

Isolated hepatocytes were prepared by collagenase digestion of the livers of male guinea pigs and used either intact or after

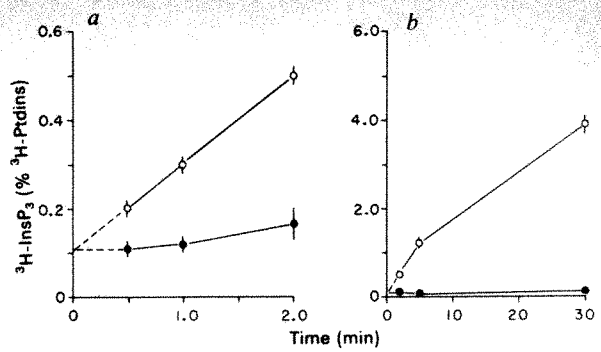


Fig. 1 Formation of ^{3}H-InsP$_3$ in guinea pig hepatocytes over 0–2 min (*a*) or 0–30 min (*b*). Freshly prepared guinea pig hepatocytes[17] were incubated in Eagle's medium with 2% bovine serum albumin (BSA) with 5 μCi ml^{-1} (three experiments) or 20 μCi ml^{-1} (three experiments) of ^{3}H-inositol, and containing 0.1 μM angiotensin II for 60 min. The cells were washed by slow centrifugation and resuspended in Eagle's medium with 2% BSA. After 5 min, 10 mM LiCl was added to block inositol 1-phosphate phosphatase[24]. In two preliminary experiments, LiCl had no discernible effect on ^{3}H-InsP$_3$ accumulation. Five minutes later, adrenaline (0.1 mM, with 10 μM (+)propranolol) was added (○); control, ●. Aliquots of the suspension were taken at various times as indicated, and were rapidly precipitated with 4.5% perchloric acid. The *p*H of the supernatant was adjusted to 8–9 with sodium borate buffer and radioactive fractions associated with specific inositol phosphates were separated by anion exchange as described previously[14,20]. For each experiment, total lipid radioactivity (almost exclusively PtdIns) was determined in two chloroform–methanol extracts, one taken just before the addition of adrenaline and one just after the last sample was taken. The radioactivity in these samples did not change significantly over the course of the experiments, nor did total tissue content of PtdIns, determined as described previously[21]. For each experiment, the cpm of ^{3}H-InsP$_3$ was expressed as a percentage of the c.p.m. of ^{3}H-PtdIns (that is, the chloroform–methanol extract). In the 5 μCi ml^{-1} experiments, samples were taken at 5 and 30 min and PtdIns radioactivity averaged 84,000 ± 9,000 c.p.m. per mg DNA; in the 20 μCi ml^{-1} experiments, samples were taken at 0.5, 1, 2 and 30 min and PtdIns radioactivity averaged 544,000 ± 30,000 c.p.m. per mg DNA. All of the data points in Fig. 1 are thus means (±s.e.m.) of three determinations except at $t = 30$ min which is the mean (±s.e.m.) of six experiments. In the latter case, the three experiments with 5 μCi ml^{-1} ^{3}H-inositol gave a value of 4.1 ± 0.3% while the 20 μCi ml^{-1} experiments gave a value of 3.7 ± 0.3%, the two not differing significantly.

permeabilization of their plasma membranes with saponin, as previously described[13]. The intact hepatocytes were incubated in Eagle's medium, while the permeable cells were incubated in a medium with high [K$^+$], low [Na$^+$], ATP (1.5 mM) and an ATP-regenerating system (see Fig. 2 legend for details). Ca-EGTA buffers were used to give free Ca^{2+} concentrations of either 180 nM or 3.3 μM (ref. 13). Formation of ^{3}H-inositol phosphates by hepatocytes preincubated with ^{3}H-inositol was measured as described previously[14].

Intact guinea pig hepatocytes showed a rapid increase in radioactivity associated with InsP$_3$ on α-adrenoceptor activation (Fig. 1). To test the hypothesis that InsP$_3$ is capable of releasing internal Ca we used a permeabilized hepatocyte preparation the characteristics of which have been described in detail elsewhere[13]. In experiments summarized in Fig. 2*a*, permeable hepatocytes were incubated in a medium with [Ca^{2+}] buffered to 180 nM in the presence of tracer quantities of ^{45}Ca. This value for [Ca^{2+}] was chosen because in these conditions the levels of phosphorylase *a*, the uptake of ^{45}Ca and the content of ^{40}Ca in saponin-treated cells were found to be similar to those in intact hepatocytes. We therefore concluded that the cytosolic [Ca^{2+}] in an intact hepatocyte is close to 180 nM[13]. Recent estimates of intracellular [Ca^{2+}] in rat[15] and guinea pig[13] hepatocytes by using the intracellular Ca-indicator 'quin-2' (ref. 16) confirm this estimate.

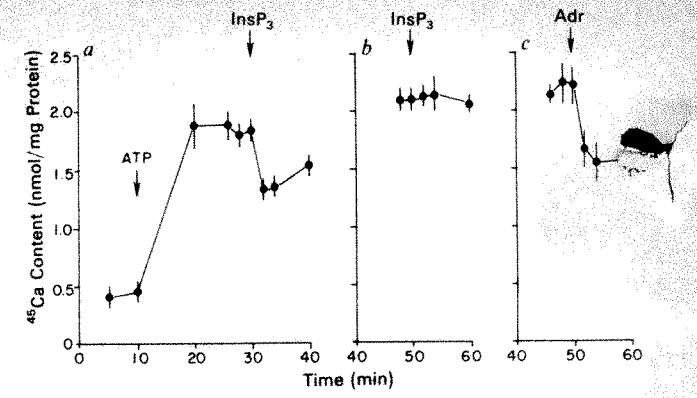


Fig. 2 *a*, The effect of InsP$_3$ (5 μM) on the ^{45}Ca content of saponin-treated hepatocytes. *b*, InsP$_3$ (5 μM) had no effect on the ^{45}Ca content of normal guinea pig hepatocytes; *c*, adrenaline (5 μM in the presence of 10 μM (+)propranolol) caused a net loss of ^{45}Ca from normal hepatocytes. In *b* and *c* the normal hepatocytes were loaded with ^{45}Ca for ~45 min (which is long enough to allow most of the intracellular Ca to exchange[13]).
Methods: At time zero ^{45}Ca was added to a suspension of saponin-treated hepatocytes incubated in a cytoplasmic-type medium[13] of the following composition (mM): NaCl, 20.0; KCl, 100; $MgSO_4$, 5.0; NaH_2PO_4, 0.96; $NaHCO_3$, 25; EGTA, 1.0, at *p*H 7.2 and 37 °C. The medium also contained albumin (2%), antimycin (10 μM) and an ATP-regenerating system consisting of creatine phosphate (5 mM) and creatine phosphokinase (5 U ml^{-1}). The [Ca^{2+}] was set at 180 nM which is believed to be [Ca^{2+}]$_i$ in a normal unstimulated guinea pig hepatocyte[13]. At 10 min, ATP (1.5 mM) was added and there was a rapid uptake of ^{45}Ca by the cells, which reached steady state by 20 min. At this [Ca^{2+}] the ATP-dependent ^{45}Ca uptake into the saponin-treated cells was insensitive to a combination of the mitochondrial poisons 2,4-dinitrophenol (0.5 mM) and oligomycin (10 μM) and is believed to be taken up mainly into the endoplasmic reticulum[13]. At 30 min, InsP$_3$ (5 μM in H_2O) was added and there was a loss of ~25% or ~0.5 nmol per mg protein of the cell ^{45}Ca content. An equivalent volume of H_2O alone had no effect on the ^{45}Ca content (not shown). ^{45}Ca content was determined in all experiments by centrifugation through ice-cold sucrose and EGTA as described previously[25]. Data were averaged ($n = 5$ in *a*, 3 in *b* and 4 in *c*) and the bars represent the s.e.m. InsP$_3$ (specifically inositol(1,4,5)trisphosphate) was prepared from human red blood cell ghosts essentially by the method of Downes *et al.*[26], the only major modification being that the rejuvenation of the red blood cells before lysis was carried out in a chloride-free medium (the chloride being replaced by citrate[26,27]). InsP$_3$ was separated on Dowex formate columns[14], and desalted by elution from a Dowex chloride column by 1 M LiCl. After addition of 100 μg mannitol, the extract was dried *in vacuo*, before removal of the LiCl with ethanol. Some of the InsP$_3$ was further purified by high-voltage ionophoresis in pyridine/acetic acid (*p*H 3.6) for 1.5 h at 40 V cm^{-1}. The InsP$_3$ was eluted from the paper, and the adjacent strip of paper was also eluted as a control. For experiments not shown here, but described in the text, inositol 1,4-bisphosphate [Ins(1,4)P$_2$] was prepared from ox brain phosphatidylinositol 4-phosphate by strong alkaline hydrolysis, and purified by paper chromatography[14]. Inositol 1-phosphate (Ins1P) and inositol 1,2-cyclic phosphate were prepared enzymatically from yeast phosphatidylinositol and purified by ionophoresis[28,29].

On addition of ATP, the permeable cells accumulated ^{45}Ca rapidly to a level of about 2 nmol per mg protein (Fig. 2*a*). Once a steady state had been achieved the addition of 5 μM InsP$_3$ to these permeable cells caused a rapid (<2 min) net release of about 0.5 nmol per mg protein of cell-associated ^{45}Ca. This fairly closely approximates the quantity of Ca initially released from intact guinea pig hepatocytes by α-adrenergic stimuli (Fig. 2*c* and ref. 17). In two experiments (not shown), 5 μM of the InsP$_3$ which was purified by high-voltage ionophoresis (see Fig. 2 legend) induced a similar release of ^{45}Ca from the saponin-treated cells, but the eluate from the adjacent strip of paper had no effect. However, 5 μM InsP$_3$ did not cause ^{45}Ca release from intact hepatocytes (Fig. 2*b*), nor

did Ca-mobilizing hormones release Ca from permeable cells (not shown). $InsP_3$ had no effect on the ATP-independent ^{45}Ca associated with the permeable cells. Inositol 1,2-cyclic phosphate (5 μM) (previously proposed as a second messenger[18]), $Ins(1,4)P_2$ (up to 10 μM), Ins1P(5 μM) and inositol (5 μM) had no effect on the ^{45}Ca accumulated in an ATP-dependent manner by permeable hepatocytes (not shown).

Figure 3 shows the concentration dependence of the response of the permeable cells to $InsP_3$ in causing the net release of ^{45}Ca which had been accumulated in an ATP-dependent manner. The EC_{50} value for this effect was 0.22 μM, and it was maximal at 5 μM.

It has previously been demonstrated that with $[Ca^{2+}]$ in the medium buffered to 180 nM, uptake of ^{45}Ca and cellular content of total Ca in permeable hepatocytes are unaffected by the mitochondrial poisons 2,4-dinitrophenol and oligomycin[13]. The ^{45}Ca released by $InsP_3$ (Fig. 2*a*) must therefore come from ATP-dependent stores other than the mitochondria, the predominant component of which is likely to be the endoplasmic reticulum[3]. To determine whether $InsP_3$ is also capable of releasing Ca from the mitochondria, the experiment in Fig. 2*a* was repeated but with the permeable cells suspended in a medium with $[Ca^{2+}]$ buffered to 3.3 μM. In these conditions a considerably larger quantity of ^{45}Ca accumulates (Fig. 4). The uptake of this ^{45}Ca can be largely blocked by dinitrophenol and oligomycin and is therefore presumed to be primarily mitochondrial[13]. As shown in Fig. 4, 5 μM $InsP_3$ produced no significant release of ^{45}Ca from the cells when $[Ca^{2+}]$ in the medium was 3.3 μM, and we conclude that mitochondrial Ca is not released by $InsP_3$, at least in these conditions and with these concentrations of Ca^{2+} and $InsP_3$. The release of the 0.5 nmol per mg protein of ^{45}Ca from the endoplasmic reticulum pool by $InsP_3$, observed at 180 nM $[Ca^{2+}]$ (Fig. 2*a*), would not be statistically detectable here because of the higher ^{45}Ca content.

These findings comprise strong evidence that $InsP_3$ could function as a second messenger linking hormone receptor activation to internal calcium release in the liver. An important question is whether the intracellular level of $InsP_3$ is elevated sufficiently during hormonal activation for it to perform this Ca-mobilizing function. Although there is no adequately sensitive and specific method for measuring the chemical levels of inositol phosphates, they can be estimated if an assumption is made regarding specific radioactivities. Since the monoester phosphates of the polyphosphoinositides turn over much more rapidly than other parts of the molecules (refs 8, 19 and unpublished observations), it is reasonable to assume that in cells labelled with ^3H-inositol the specific radioactivities of the three phosphoinositides and thus also their breakdown products, the inositol phosphates, will all be similar[20]. In each of the experiments shown in Fig. 1, therefore, the radioactivity associated with phosphatidylinositol (PtdIns) (which did not change appreciably during the experiments and was not significantly altered by hormones) was measured and the radioactivity associated with $InsP_3$ expressed as a percentage of that value. From these data, and the knowledge that guinea pig hepatocytes contain 37.1 ± 2.7 ($n = 5$) nmol per mg protein (assayed as described previously[21]) of PtdIns, the cellular content of $InsP_3$ could be calculated. Assuming that hepatocytes contain 2.2 μl cell water per mg protein[6], the net increase in cellular $InsP_3$ induced by a maximal concentration of adrenaline was estimated to be about 16 μM at 30 s, 32 μM at 1 min and 53 μM at 2 min. As the α-adrenoceptor-induced release of ^{45}Ca from isolated intact guinea pig hepatocytes is not maximal until 2 min (see Fig. 2 and ref. 17), the $InsP_3$ would seem to be formed rapidly enough to act as a second messenger.

When applied to permeable cells in concentrations even lower than these estimated values, and with free $[Ca^{2+}]$ buffered to a level similar to that in the cytosol, $InsP_3$ induced the release of Ca from an intracellular store. Further, the quantity of Ca was similar to that released from the intracellular stores of intact hepatocytes by hormones. These results also support the view[13] that the primary source of hormone-sensitive Ca in the liver is a non-mitochondrial pool, perhaps the endoplasmic reticulum.

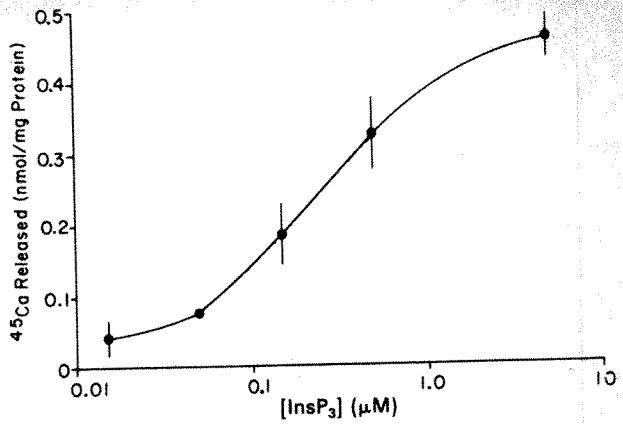


Fig. 3 The concentration–effect relationship for the ability of $InsP_3$ to release ^{45}Ca from permeable hepatocytes. Experiments were carried out as described for Fig. 2*a* and the amount of ^{45}Ca released was determined at 2 min by which time the effect was maximal. ^{45}Ca contents were determined by the rapid dilution of 100-μl aliquots of cell suspension in 10 ml of ice-cold isotonic sucrose containing 4 mM EGTA and tracer amounts of ^3H-sucrose for determination of trapped volume. The sample was then filtered rapidly through GF/C filters, washed with 10 ml of ice-cold isotonic sucrose, and the filters were counted for radioactivity. Data were averaged and the bars indicate s.e.m. for between three and five observations except at 5 μM where $n = 13$ and which includes data for which the ^{45}Ca content was determined by centrifugation[25] (from Fig. 2*a*).

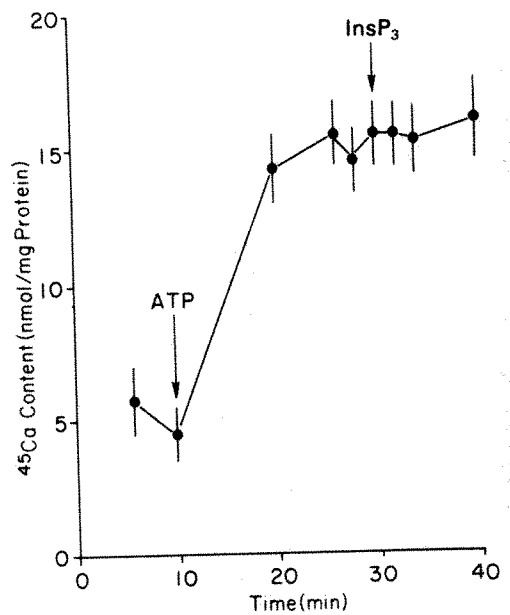


Fig. 4 Effect of $InsP_3$ (5 μM) on the ^{45}Ca content of saponin-treated guinea pig hepatocytes. The saponin-treated hepatocytes were incubated as described for Fig. 2*a* except that the $[Ca^{2+}]$ of the cytoplasmic-type medium was increased to 3.3 μM, a concentration at which there is substantial 2,4-dinitrophenol (0.5 mM)-and oligomycin (10 μM)-sensitive ^{45}Ca uptake, presumably by the mitochondria[13]. The ATP-dependent ^{45}Ca uptake was much greater than in Fig. 2*a* and as the endoplasmic reticulum mechanism saturates at about 3 nmol per mg protein[13], most of the uptake is ATP-dependent uptake into mitochondria. Application of $InsP_3$ had no effect on the ^{45}Ca content of the cells. Data were averaged and the bars indicate s.e.m. of three observations.

In the liver, as in many other tissues, agonist-induced release of intracellular Ca is accompanied by an increased membrane permeability to Ca (ref. 6). Following inositol lipid breakdown, the resulting diacylglycerol is rapidly phosphorylated to phosphatidic acid which it has been suggested may mediate Ca entry by functioning as a Ca-ionophore (ref. 22 but see ref. 23).

Although not addressed specifically by these studies, the possibility must now be considered that $InsP_3$ could also regulate plasma membrane permeability to Ca by the same, as yet unknown, mechanism involved in the release of intracellular Ca.

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Cyclic nucleotides control a system which regulates Ca^{2+} sensitivity of platelet secretion

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Cellular responses to extracellular signals are mediated by changes in the intracellular concentrations of one or more second messengers[1]. In platelets, inhibitory agonists increase intracellular cyclic-3′,5′-AMP ([cyclic AMP]$_i$ (refs 2, 3)) whereas excitatory agonists increase [Ca^{2+}]$_i$ and/or [1,2-diacylglycerol]$_i$ (refs 4–9), and in some cases decrease [cyclic AMP]$_i$ (refs 10, 11). Both activation and inhibition of platelet responses have been attributed to an increase in [cyclic-3′,5′-GMP]$_i$ (refs 8, 12). The activity of protein kinase C, which is associated with the platelet secretory response, is increased by both 1,2-diacylglycerol and Ca^{2+} (refs 4, 7, 8). The role of cyclic AMP may involve either inhibition of Ca^{2+} mobilization to the cytosol[13] or stimulation of intracellular Ca^{2+} uptake[14], and in addition inhibition of 1,2-diacylglycerol formation[15,16]. The relationship between cyclic-3′,5′-GMP (cyclic GMP) and other second messengers in platelet activation has not been defined. Using platelets made permeable by exposure to an intense electric field[17,18], we demonstrate here modulation of the Ca^{2+} sensitivity of platelet secretion by thrombin, and by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and 1-oleyl-2-acetylglycerol (OAG), both potent activators of protein kinase C. The effect of thrombin is selectively modified by cyclic GMP and cyclic AMP. The response to OAG and TPA is also modulated by cyclic AMP but to a much lesser extent.

An increase in [Ca^{2+}]$_i$ has previously been shown to induce ^{14}C-serotonin secretion from permeabilized platelets[19]. Addition of thrombin or a synthetic activator of protein kinase C such as OAG[20] or TPA[21,22], increases the sensitivity of the secretory response to Ca^{2+} by shifting the dose–response curve to the left (Fig. 1). In addition, thrombin, TPA and OAG cause a small increase in the extent of secretion at 1 nM Ca^{2+}. Although this increase could indicate the existence of a secretory mechanism which is not controlled by Ca^{2+} (refs 6, 7), its contribution to the overall secretory response is minimal in the conditions used here. Since both TPA and OAG mimic the effect of thrombin (Fig. 1), these data strongly support the proposed role of protein kinase C in amine storage granule secretion[7,8]. A similar increase in the sensitivity of the secretory

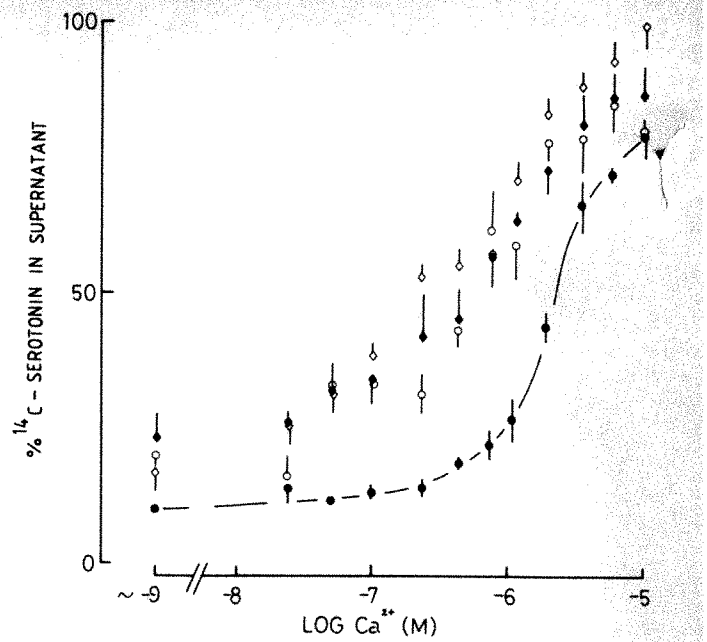


Fig. 1 The Ca^{2+} sensitivity of ^{14}C-serotonin secretion from platelets in the absence (●) and in the presence of thrombin (◇), OAG (○) or TPA (◆). Human platelets were prepared in the presence of 0.1 mM acetysalicylate as described previously[18], and were suspended in medium containing (in mM): glycine, 280; K-glutamate, 10; ATP, 5; Mg-acetate, 7; PIPES, 20; EGTA, 1; *p*H 6.7. The platelets were rendered permeable by 10 exposures to an intense electric field (20 kV cm^{-1}, $\tau \sim 30$ μs)[17]. After incubation for 15 min at 22 °C, 1-ml aliquots of the suspension (~2×10^8 cells ml^{-1}) were added to Ca-EGTA buffers and exactly 40 s later 0.2-ml aliquots of these Ca-challenged cells were added to 0.02 ml of thrombin, OAG, TPA or a control suspending medium. The final concentrations of EGTA, thrombin, OAG and TPA in the cell suspension were 16 mM, 0.5 U ml^{-1}, 30 μg ml^{-1} and 10 ng ml^{-1}, respectively. After incubation for 5 min at 22 °C the platelets were removed by centrifugation at 8,000*g* for 2 min and 0.05-ml aliquots of the supernatant fraction assayed for ^{14}C content by liquid scintillation counting. The time taken to challenge the cells with all the Ca buffers was 10 min, with the order of Ca^{2+} challenges being random. The ordinate shows the amount of ^{14}C in the supernatant fraction expressed as a percentage of the total ^{14}C measured by counting aliquots of the cell suspension. The data points refer to the mean of four determinations with the bar showing the s.e.m. OAG and TPA were prepared as stock solutions (60 mg ml^{-1} and 10 μg ml^{-1}, respectively) in dimethyl sulphoxide (DMSO), dispersed in the suspending medium by sonication under N_2 for 5 s, then diluted appropriately in that medium. The final concentration of DMSO in the cell suspension was 0.1% (v/v); control experiments showed that this level of DMSO had no effect on the secretory response and that addition of thrombin, OAG and TPA caused no significant increase in the extent of cellular lysis as measured by release of lactate dehydrogenase. Ca^{2+} concentrations were calculated using equilibrium constants of $10^{-6.127}$M (Ca-EGTA), $10^{-1.362}$M (Mg-EGTA), $10^{-3.50}$M (Ca-ATP) and $10^{-3.64}$M (Mg-ATP)[34]. The EC_{50} value for Ca^{2+} is 2.0 μM in the absence of other additions and 0.3–0.5 μM in the presence of 0.5 U ml^{-1} thrombin, 10 ng ml^{-1} TPA or 30 μg ml^{-1} OAG.

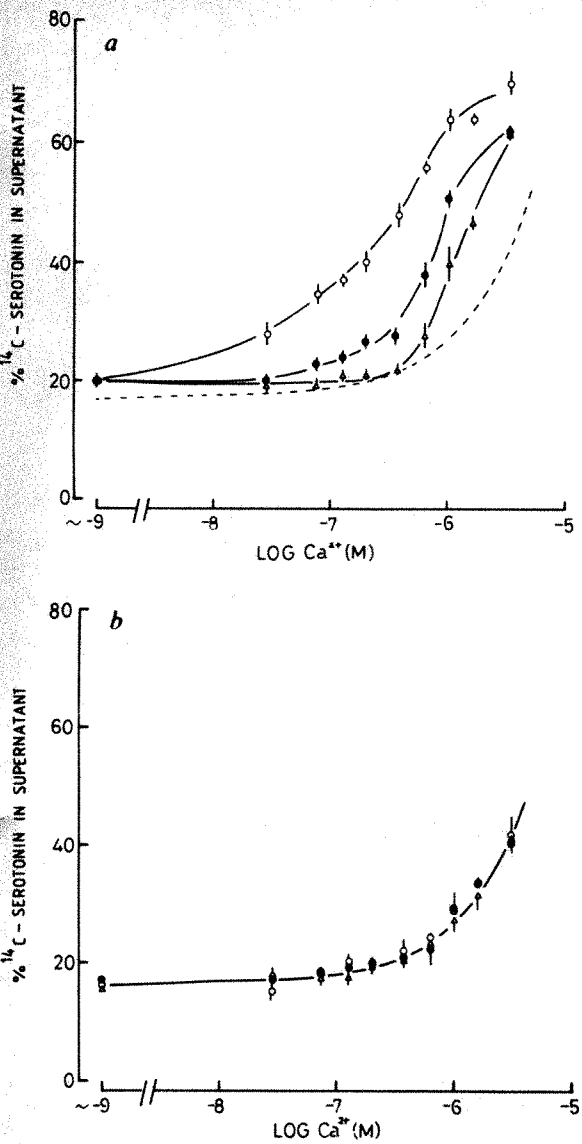


Fig. 2 The effect of cyclic AMP and cyclic GMP on the Ca^{2+} sensitivity of ^{14}C-serotonin release in the presence (*a*) or absence (*b*) of thrombin. Platelets were prepared and rendered permeable as described for Fig. 1 except that the suspending medium contained 1 mM ATP, 3 mM Mg-acetate, 0.5 mM EGTA and at a *p*H of 6.6. After incubation for 25 min at 22 °C, aliquots of the permeabilized suspension ($\sim 4 \times 10^8$ cells ml^{-1}) were incubated for 5 min at 22 °C with 0.2 μM 3-isobutyl-1-methylxanthine (IBMX) and 1 mM bis(*O*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*-tetraacetic acid (BAPTA) either alone (●), in the presence of 20 μM cyclic AMP (△) or in the presence of 20 μM cyclic GMP (○). 0.1-ml aliquots of these suspensions were added to 0.1 ml of the suspending medium containing Ca-EGTA buffers either alone (*b*) or in the presence of thrombin (*a*). The final concentrations of EGTA and thrombin were 20 mM and 0.5 U ml^{-1}, respectively. The system was incubated for 5 min at 22 °C, centrifuged and the supernatant fraction assayed for ^{14}C content as described for Fig. 1. Data points refer to the mean of four determinations with the error bars showing the s.e.m. When Ca-EGTA buffers were added to the cell suspension containing BAPTA, the *p*H increased from 6.6 to 6.7. The Ca^{2+} concentrations stated above were calculated from the Ca-EGTA stability constants at these *p*Hs[34], and from the stability constants for Ca-BAPTA and Mg-BAPTA of $10^{-6.959}$ M and $19^{-1.77}$ M, respectively[35]. The curve drawn through the data points of cells incubated in the absence of thrombin (*b*) is also included as a dashed line in *a* to facilitate comparison with the data obtained in the presence of thrombin ± the cyclic nucleotides. The EC_{50} values for Ca^{2+} obtained from these data are 2.0 μM (no additions), 0.5 μM (in the presence of 0.5 U ml^{-1} thrombin), 1.2 μM (in the presence of 0.5 U ml^{-1} thrombin + 20 μM cyclic AMP) and 0.15 μM (in the presence of 0.5 U ml^{-1} thrombin + 20 μM cyclic GMP).

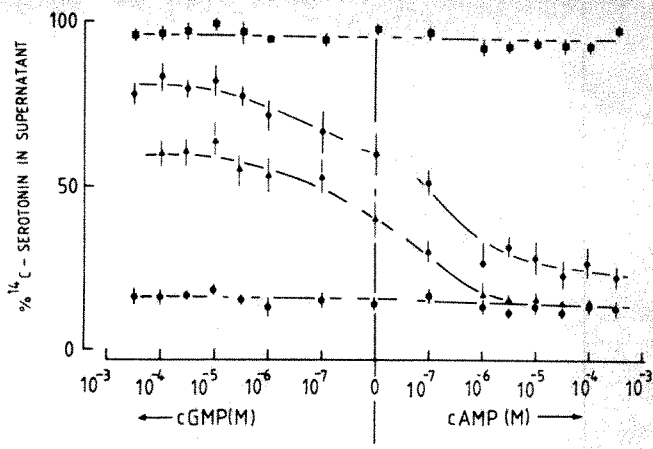


Fig. 3 Dose–response curves for the effects of cyclic AMP and cyclic GMP on ^{14}C-serotonin secretion mediated by thrombin in the presence of four fixed Ca^{2+} concentrations. Platelets were prepared and suspended in the medium described for Fig. 1 except that 1 mM BAPTA was added instead of 1 mM EGTA, and the *p*H of the medium was 6.6. The cells were rendered permeable as described for Fig. 1, then incubated for 8–10 min at 22 °C and at a cell density of 4×10^8 cells ml^{-1} in the presence of 5 μM IBMX and at concentrations of either cyclic AMP or cyclic GMP as shown. 0.1-ml aliquots of these suspensions were then added to 0.1 ml of the suspending medium containing thrombin and Ca-EGTA buffers to give final Ca^{2+} concentrations (calculated) of ~1 nM (●), 0.15 μM (▲), 0.5 μM (◆) and 5.0 μM (■). The final concentrations of thrombin and EGTA were 0.7 U ml^{-1} and 7.7 mM, respectively. After incubation for 5 min at 22 °C the platelets were removed and the supernatant fraction assayed for ^{14}C content as described for Fig. 1. The data points are the mean of four determinations, with error bars showing the s.e.m. The final Ca^{2+} concentrations were calculated as described for Fig. 2. No significant enhancement of the response was observed at 0.15 μM or 0.5 μM Ca^{2+} when 20 μM cyclic-2′3′-GMP was used in place of cyclic-3′5′-GMP.

response to Ca^{2+} has been observed on addition to TPA to permeabilized adrenal medullary cells[23]. The data also explain the previously reported[24] difference in secretory behaviour between intact and permeabilized platelets, and implicate 1,2-diacylglycerol as the factor responsible for the enhancement by thrombin of ^{14}C-serotonin secretion[25]. Production of 1,2-diacylglycerol on stimulation by thrombin has been observed in both intact[9] and permeabilized (R. J. Haslam, personal communication) platelets, although in the latter preparation the expected phosphoinositide breakdown[26] has not yet been demonstrated[27].

Addition of 20 μM cyclic AMP or cyclic GMP has no significant effect on the dose–response curve for ^{14}C-serotonin secretion induced by addition of Ca^{2+} when added alone (Fig. 2*b*). However, in the presence of thrombin (Fig. 2*a*), addition of 20 μM cyclic AMP depresses the response to a level approaching that observed in the presence of Ca^{2+} alone by shifting the dose–response curve to the right, whereas addition of 20 μM cyclic GMP enhances the response to Ca^{2+} by shifting the dose–response curve to the left (Fig. 2*a*). Dose–response curves obtained for the effects of cyclic AMP and cyclic GMP determined in the presence of thrombin and at four fixed calculated Ca^{2+} concentrations (Fig. 3) provide further evidence for specificity. Neither cyclic nucleotide has any significant effect at very low or saturating Ca^{2+} concentration where addition of thrombin causes little, if any, increment in ^{14}C-serotonin secretion (Figs 1, 3). At intermediate Ca^{2+} concentrations the presence of cyclic AMP markedly inhibits ^{14}C-serotonin secretion, with half-maximal inhibition obtained at or below 10^{-6} M (Fig. 3) whereas addition of cyclic GMP enhances ^{14}C-serotonin secretion with an EC_{50} in the micromolar range (Fig. 3). This effect of cyclic-3′5′-GMP is not mimicked by addition of cyclic-2′3′-GMP (Fig. 3 legend).

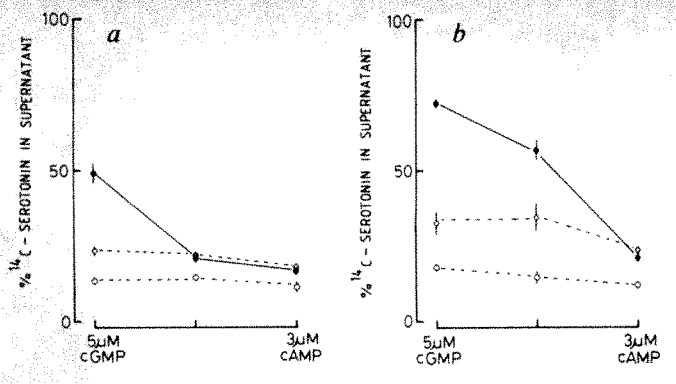


Fig. 4 Effect of raising the OAG and thrombin concentrations at fixed calculated Ca^{2+} concentrations of 0.17 μM (*a*) and 0.4 μM (*b*) in the presence and absence of cyclic AMP and cyclic GMP. Platelets were prepared and rendered permeable as described for Fig. 2. After incubation for 25 min at 22 °C the permeabilized platelet preparations ($\sim 3 \times 10^8$ cells ml^{-1}) were incubated for a further 5 min at 22 °C with 20 mM Ca-EGTA buffers corresponding to 0.17 μM Ca^{2+} (*a*) and 0.4 μM Ca^{2+} (*b*). 1.2-ml aliquots of the cell suspensions were then incubated for a further 5 min at 22 °C with 0.2 μM IBMX in the absence or presence of 3 μM cyclic AMP or 5 μM cyclic GMP. 0.1-ml aliquots of these suspensions were then added to 0.05 ml of a cell suspension medium containing the same Ca-EGTA buffers, in the absence of other additions (○) or in the presence of OAG (◇) or thrombin (◆). The final concentrations of thrombin and OAG were 0.6 U ml^{-1} and 7.5 μg ml^{-1}, respectively. The cell suspensions were incubated for 5 min at 22 °C before being centrifuged, and the supernatant fraction was assayed for ^{14}C content as described for Fig. 1. Data points refer to the mean of four determinations, with the error bars showing the s.e.m. The difference in the relative extents of the responses to thrombin and OAG as compared with their similarity in Fig. 1 results from the use of different OAG concentrations in these two experiments.

Studies similar to those shown in Fig. 2 have been performed using OAG instead of thrombin. Addition of cyclic AMP (20 μM) reduces the extent to which OAG shifts the dose–response curve for activation by Ca^{2+} to the left. However, the effect of cyclic AMP on the Ca^{2+}-dependent response to OAG or TPA occurs at a much higher concentration ($IC_{50} > 10$ μM) than that which inhibits the response to thrombin ($IC_{50} = 0.3$ μM) (data not shown but see Fig. 4). The effect of these cyclic nucleotides on the secretory response to thrombin or OAG has also been examined in conditions which partially simulate conditions in the intact cell. At a Ca^{2+} concentration of 0.17 μM, which approaches that typical of unstimulated intact platelets[5], addition of thrombin or OAG causes little increase in the secretory response. This small response is not obviously inhibited by 3 μM cyclic AMP. The response to thrombin, but not to OAG, is enhanced by addition of 5 μM cyclic GMP (Fig. 4*a*), in accord with the ability of cyclic GMP to enhance only the increase in the Ca^{2+} sensitivity of thrombin-induced secretion (Fig. 2 and data not shown). At a higher Ca^{2+} concentration (0.4 μM) which is still below the threshold for induction of shape change by ionomycin[5], a significant secretory response results from addition of either thrombin or OAG (Fig. 4*b*). At this higher Ca^{2+} concentration the response to thrombin is further enhanced in the presence of 5 μM cyclic GMP but is clearly inhibited in the presence of 3 μM cyclic AMP. The response to OAG is, in contrast, unaffected by the presence of cyclic GMP and is only slightly reduced by cyclic AMP (Fig. 4*b*).

These data therefore suggest that cyclic nucleotide control is primarily, although not exclusively, exerted before the formation of 1,2-diacylglycerol. Our data provide strong support for inhibition of phospholipase C by cyclic AMP, which has previously been proposed on the basis of data obtained using dibutyryl cyclic AMP and intact platelets[15,16]. This inhibitory effect may be exerted directly on phospholipase C or on a factor which regulates the supply of substrate to this enzyme. However, another inhibitory site for cyclic AMP lies beyond phospholipase C, on the stimulus–response coupling pathway, but becomes important only at higher cyclic AMP concentrations. The effect of cyclic GMP is, in contrast, restricted to enhancement of the secretory response induced by thrombin at submaximal levels of Ca^{2+} (Figs 2–4), and may therefore result wholly from direct or indirect activation of phospholipase C. Such an effect is inconsistent with results obtained using intact platelets in which an increase in [cyclic GMP]$_i$ has been reported to inhibit secretion and 1,2-diacylglycerol formation[8,28,29] but accords with a study in which addition of dibutyryl cyclic GMP enhanced platelet aggregation and secretion[12]. The reason for the discrepancy with the former studies is unclear, although the *p*H and temperature used here differ substantially from the conditions in which the response of the intact cell is usually measured. However, the intact cell data may be equivocal as certain agents, for example, sodium nitroprusside, which increase [cyclic GMP]$_i$, also less effectively raise [cyclic AMP]$_i$ (ref. 28), while others, such as ascorbic acid, fail to inhibit the aggregatory response[30]. Furthermore, inhibition of responses by 8-bromo-cyclic GMP[8,28,29] is not definitive because in other systems the effects of membrane-permeant cyclic nucleotide derivatives on intact cells have not always been consistent with results obtained by direct introduction of cyclic nucleotides into the cytosol[31].

Finally, if inositol-1,4,5-trisphosphate, another product of the breakdown of triphosphoinositide by phospholipase C, causes mobilization of Ca^{2+} to the cytosol as described for the pancreatic acinar cell[32,33], control of this enzyme by cyclic nucleotides could provide coordinate regulation of Ca^{2+} mobiliza[illegible] and 1,2-diacylglycerol formation in the platelet.

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Dihydropyridine BAY-K-8644 activates chromaffin cell calcium channels

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Douglas and Rubin[1] suggested that "the role of acetylcholine as a transmitter at the adrenal medulla is to cause some brief change in medullary cells which allows Ca ions to penetrate them and trigger the catecholamine ejection process". The Ca^{2+}-channel blocking agents, verapamil, nifedipine and nitrendipine, have been used widely to investigate the properties of slow Ca^{2+} channels in a variety of tissues[2], including the adrenomedullary chromaffin cell[3,4]. Recently, small modifications to the nifedipine molecule produced a derivative, BAY-K-8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate), that in contrast to the Ca^{2+}-channel blocking agents, stimulated cardiac and vascular smooth muscle contractility[5]. We have tested whether this compound behaves as a Ca^{2+}-channel activator at the chromaffin cell membrane as shown by Schramm *et al.*[5] in smooth muscle cells. The experiments described here strongly suggest that it does so.

Cat adrenal glands were isolated and prepared for retrograde perfusion with Krebs-bicarbonate solution at room temperature[6]. After 1 h initial perfusion with oxygenated Krebs-bicarbonate solution, spontaneous catecholamine outputs from cat adrenal glands amounted to 187 ± 24 ng per 10 min ($N = 75$). When the glands were subsequently stimulated for 2 min, at 30-min intervals, with isotonic solutions containing increasing concentrations of K^+, the net release of catecholamines was directly proportional to the concentration of K^+ used up to 59 mM. BAY-K-8644 at concentrations of 10^{-8}–10^{-4} M did not affect spontaneous catecholamine output; in the presence of BAY-K-8644 (1 μM), the release was 145 ± 28 ng per 10 min ($N = 42$). A drug concentration of 1 μM markedly increased catecholamine release evoked by 17.7 mM K^+; while evoked net release by K^+ was 2.93 ± 0.38 μg per 8 min ($N = 27$), in the presence of 1 μM BAY-K-8644 it was 10.1 ± 1.51 μg per 8 min ($N = 14$). Secretory responses to 59 mM K^+ were not affected by the drug. These results suggest that BAY-K-8644 is not acting on quiescent chromaffin cells and that a moderate depolarizing stimulus is required for the drug to manifest its potentiating effects on secretion. If this agent were acting on Ca^{2+} channels, the lack of potentiation of the secretory responses to higher K^+ concentrations might indicate that in these conditions, all channels are in their maximal state of activation or their most inactivated form.

Since the potentiation by BAY-K-8644 was greater at moderately depolarizing stimuli, 17.7 mM K^+ solutions were used to test the effects of varying concentrations of the drug on K^+-evoked catecholamine release. Six 2-min pulses of K^+ were given to perfused cat adrenal glands at 20-min intervals; moderate catecholamine release rates were obtained. The release was slowly decaying from the first to the sixth pulse, the amount of catecholamines found in S_6 being about 50% of that obtained in S_1 (Fig. 1*a*). In the contralateral paired gland, BAY-K-8644 started to potentiate catecholamine release at 10^{-8} M, and had already reached maximum potentiation at 10^{-7} M (Fig. 1*a*); at 10^{-6} and 10^{-5} M the potentiating effect was similar, but at 10^{-4} M the secretory response to K^+ fell abruptly, probably because at these high concentrations the drug behaves as a Ca^{2+}-channel blocking agent. The great potentiation obtained with low concentrations of BAY-K-8644 suggests a highly selective effect; since high K^+ is presumed to activate catecholamine secretion by directly depolarizing the chromaffin cell and opening voltage-sensitive Ca^{2+} channels[3,7,8], it seems feasible to assume that the target receptor for the drug is the slow Ca^{2+} channel. The fact that nitrendipine (a Ca^{2+}-channel blocking agent that potently inhibits catecholamine release evoked by nicotine or high K^+)[3] antagonizes the potentiating effect of BAY-K-8644 on K^+-evoked secretion (Fig. 1*b*), is consistent with this conjecture.

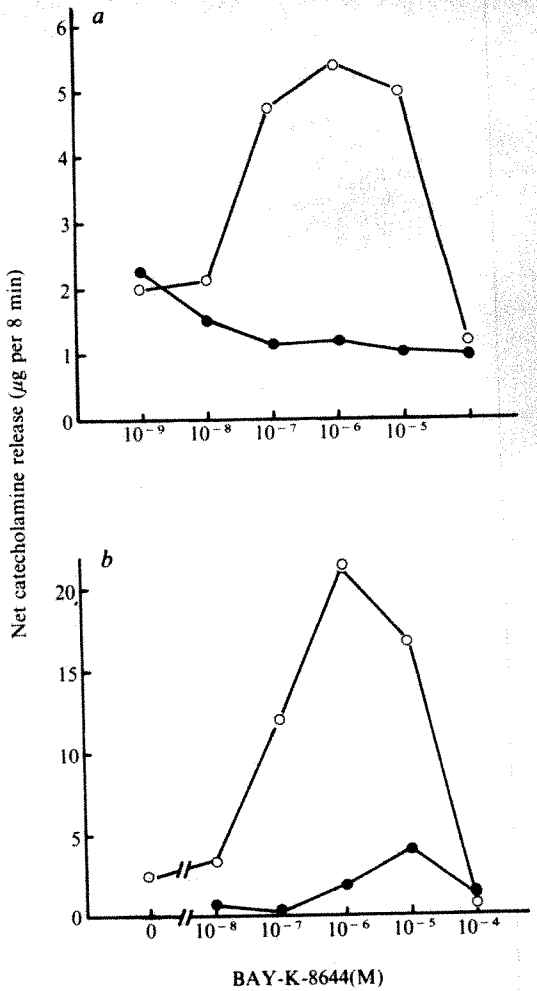


Fig. 1 Effects of increasing concentrations of BAY-K-8644 on the net release of catecholamines evoked by repeated 2-min pulses of 17.7 mM K^+ (NaCl substituted by equiosmolar amounts of KCl), given to perfused cat adrenal glands at 30-min intervals. *a*, Six pulses of K^+ were given in the absence or presence of increasing concentrations (abscissa) of BAY-K-8644. These are the results of a paired typical experiment (out of four) in which one gland was free of drug (●) and the contralateral gland was perfused with 10^{-9}–10^{-4} M BAY-K-8644 in a cumulative manner (○); each concentration of drug was added 10 min before, during the K^+ pulse and until the following concentration was introduced. *b*, A paired experiment; one gland was perfused with BAY-K-8644 at the concentrations shown on the abscissa (○); the contralateral gland was perfused in addition to this drug, with the Ca^{2+} channel antagonist nitrendipine (10^{-6} M present 10 min before the first K^+ pulse and during the rest of the experiment) (●). Total catecholamine content of perfusate samples (noradrenaline plus adrenaline) was determined without further purification on alumina[18]. Net catecholamine release during high K^+ was calculated by subtracting the basal, spontaneous release from the release obtained in the 2-min high-K^+ sample plus that obtained in three additional 2-min samples and expressed as μg per 8 min.

If BAY-K-8644 activates Ca^{2+} channels, it should favour the influx of ^{45}Ca by the chromaffin cell; this was certainly the case, as shown in the experiments of Fig. 2. The uptake of ^{45}Ca by acutely isolated chromaffin cells suspended in oxygenated Krebs-bicarbonate solution was substantially increased in the

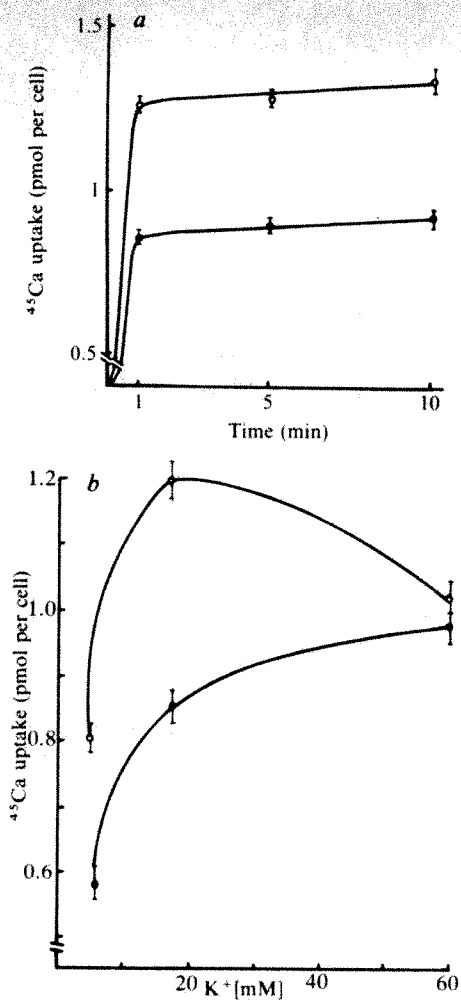


Fig. 2 BAY-K-8644 increases ^{45}Ca uptake into acutely isolated bovine adrenal chromaffin cells prepared as previously described[19] (○, BAY-K-8644; ●, control). *a*, Time course of the uptake of ^{45}Ca by chromaffin cells exposed to high K^+ (17.7 mM) solution in the absence (control) or the presence of BAY-K-8644 (1 μM). Inmediately after isolation and washing, cells were incubated in oxygenated Krebs-bicarbonate solution at 37 °C for 15 min; then aliquots of the cell suspension containing 5×10^5 cells were added to 1 ml Krebs solution with 17.7 mM K^+ (NaCl reduced equiosmotically) and 3×10^6 c.p.m. ^{45}Ca per assay (Amersham; specific activity 20.1 mCi mg^{-1}). In BAY-K-8644-treated cells, the drug (1 μM) was present 15 min before adding K^+ and ^{45}Ca. One, five or ten minutes after adding ^{45}Ca and K^+, 3 ml Krebs solution (with or without BAY-K-8644) were added and the cells centrifuged at 2,000 r.p.m. for 10 min; cells were resuspended and washed four times in the above solution. Finally, they were vigorously resuspended in 2 ml of 0.4 M perchloric acid and centrifuged at 2,000 r.p.m. for 10 min; 0.5 ml of the supernatants were added to 5 ml Instagel (Packard) and the radioactivity was counted in a LS2800 Beckman scintillation counter. ^{45}Ca taken up and retained by cells was expressed as pmol per cell (ordinate). Data are means ± s.e. of eight experiments. *b*, Effects of BAY-K-8644 on ^{45}Ca uptake by chromaffin cells suspended in solutions containing 5.9 (normal Krebs), 17.7 or 59 mM K^+. BAY-K-8644 (1 μM) was present 15 min before and during K^+ stimulation. Conditions were as above. Results are means ± s.e. of eight experiments.

presence of 17.7 mM K^+, in relation to the uptake observed in non-stimulated cells; uptake was almost complete after 1 min incubation, although it continued to rise slightly after 5 or 10 min incubation (Fig. 2*a*). When cells were suspended in the presence of BAY-K-8644 at a concentration (10^{-6} M) which maximally potentiated K^+-evoked catecholamine release (Fig. 1*a*), ^{45}Ca uptake–retention in the presence of 17.7 mM K^+ was enhanced at all incubation times studied (Fig. 2*a*). Figure 2*b* shows the uptake of ^{45}Ca in the presence of 17.7 or 59 mM K^+; BAY-K-8644 increased ^{45}Ca influx significantly ($P < 0.001$, $N = 8$) at

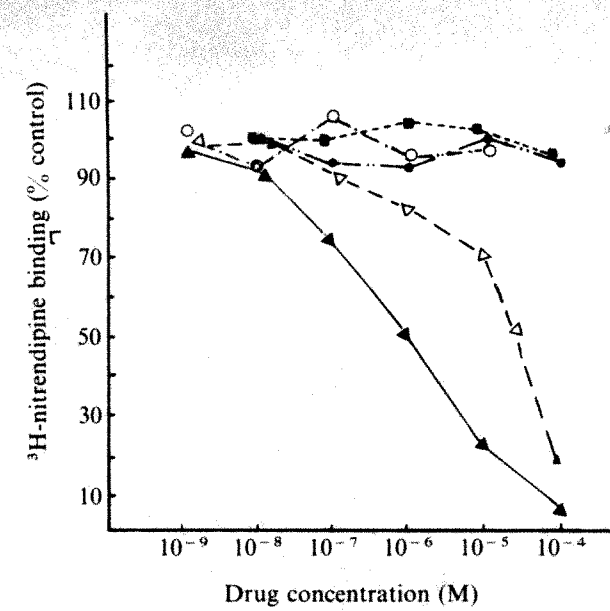


Fig. 3 Specific binding of ^{3}H-nitrendipine to bovine adrenomedullary membrane fragments and its displacement by BAY-K-8644. A crude microsomal fraction isolated from bovine adrenal medulla homogenates (0.3 M sucrose containing 10 mM Tris buffer, *p*H 7.4) was used as the source of membranes. The reaction mixture consisted of 300 μg membrane protein, 50 mM Tris buffer *p*H 7.4, 1 nM ^{3}H-nitrendipine and increasing concentrations of displacing agent (abscissa). Incubation was at 37 °C under a sodium lamp for 30 min. The ordinate shows ^{3}H-nitrendipine specifically bound to membranes as % of maximal binding in the absence of displacing agent. IC_{50} values for nitrendipine and BAY-K-8644 were, respectively, 1 and 50 nM. Data are means ± s.e. of three to six experiments. ○, Imipramine; ●, cocaine; ■, nicotine; △, BAY-K-8644; ▲, nitrendipine.

the lower K^+ concentration but not when 59 mM K^+ was used, suggesting once more that at higher K^+ concentrations, Ca^{2+} channels might be fully activated and that ^{45}Ca entry through them cannot be further increased. Note that BAY-K-8644 also slightly but significantly enhanced the ^{45}Ca taken up by quiescent cells ($P < 0.01$, $N = 8$); this finding contrasts with the lack of effect of the drug on spontaneous catecholamine release or rabbit aorta contractility[5]. Even so, these experiments support the suggestion that this agent is promoting Ca^{2+} uptake thr specific voltage-sensitive Ca^{2+} channels.

^{3}H-nitrendipine has been used as a specific radioliga characterize the dihydropyridine receptor probably asso with the Ca^{2+} channel in various tissues[9] including the a medulla[10]. Since nitrendipine antagonized the potentiating effects of BAY-K-8644 on catecholamine release evoked by high K^+, the possible modification of ^{3}H-nitrendipine binding to membranes could help to define further its presumed binding site on the chromaffin cell membrane and potentiation of K^+-evoked catecholamine release. Measurements of ^{3}H-nitrendipine-specific binding to membrane fragments from bovine adrenal medulla demonstrate a saturable binding with an apparent K_D of 1.18 ± 0.32 nM and a B_{max} of 325.4 ± 136 fmol per mg protein ($N = 4$). These values are compatible with the presence in the chromaffin cell membrane of a high-affinity binding site probably associated with the voltage-dependent Ca^{2+} channel. The specificity of the radioligand for the Ca^{2+} channel is further corroborated by the fact that agents which bind to the acetylcholine site (nicotine) or to the ionophoric site (imipramine, cocaine)[3] of the nicotinic cholinoceptor did not affect ^{3}H-nitrendipine binding (Fig. 3). In contrast, BAY-K-8644 fully displaced ^{3}H-nitrendipine bound to membranes, suggesting once more that this drug is acting on the voltage-sensitive Ca^{2+} channel.

Studies involving measurements of catecholamine release

evoked by depolarizing agents[3,11–13], and monitoring of ^{45}Ca transmembrane fluxes[14–16] or electrical recordings using patch-clamp techniques[17] have indicated the presence in the chromaffin cell membrane of slow Ca^{2+} channels which are sensitive to voltage changes. The potency of nitrendipine in inhibiting[3] and that of BAY-K-8644 in enhancing K^+-evoked catecholamine release suggests that the chromaffin cell membrane might contain a 'dihydropyridine' receptor closely related to this voltage-sensitive slow Ca^{2+} channel; if so, it is likely that an endogenous modulator for such a receptor might be manufactured by the cell to control the activation and inactivation of Ca^{2+} channels either from the cytosol or from the cell exterior after its exocytotic release, as suggested for smooth muscle cells[5]. In any case, the dihydropyridine BAY-K-8644 might be the first compound available to manipulate pharmacologically the rates of activation or inactivation of Ca^{2+} channels; if so, it will prove a useful tool for studying the properties of such channels as well as their functional dynamics during the secretory cycle for catecholamines and other neurotransmitters and hormones.

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A haemoglobin switching activity modulates hereditary persistence of fetal haemoglobin

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During human development there is a switch from fetal to adult haemoglobin formation, reflecting the differential expression of fetal ($^G\gamma$ and $^A\gamma$) and adult (β and δ) globin genes. Mutations that inhibit this switch produce variants of the syndrome of hereditary persistence of fetal haemoglobin (HPFH). Adult heterozygotes for these mutants produce 15–30% fetal haemoglobin (HbF) in their red cells[1]. The general assumption is that the mutations result in a permanent switching on of γ-globin genes. Here, however, we show that fetal globin expression can be turned off in cultures of HPFH cells by an uncharacterized factor in fetal sheep serum. This is the first demonstration that mutations affecting the developmental expression of globin genes can be modulated by exogenous factors. The findings raise the possibility that the phenotype of HPFH is not simply the direct result of mutations in or around globin genes but the consequence of the mutations on the interaction of globin genes with *trans*-acting regulatory factors.

The studies described here followed the observation that an activity (named haemoglobin switching activity[2–4]) present in sera from sheep fetuses has profound effects on HbF expression in cultures of human erythroid cells. This activity (the nature of which remains unknown) turns off HbF synthesis in cultures of erythroid progenitors from normal adults, from persons with activated HbF synthesis *in vivo*, and from patients with sickle cell anaemia or a β-thalassaemia syndrome[2–4]. The activity induces switching of HbF to HbA synthesis in neonatal erythroid progenitor cultures, but fails to induce HbF to HbA switching in cultures of fetal burst-forming units (BFU-E)[2–4]. *In vitro* studies suggest that haemoglobin switching occurs through a direct interaction with early as well as late erythroid progenitors[3].

The availability of culture conditions that can modify HbF expression has enabled us to study HPFH mutations. HPFH mutations with *cis* $^G\gamma$ and $^A\gamma$ but not β and δ ($^G\gamma^A\gamma$ HPFH) globin expression or *cis* $^G\gamma$ ($^G\gamma$ HPFH) or $^A\gamma$ ($^A\gamma$ HPFH) chain synthesis are known[1]. Previous structural analyses have revealed various deletions in the β-globin genomic regions of $^G\gamma^A\gamma$ HPFH mutants but no globin gene abnormalities in the $^G\gamma$ or $^A\gamma$ HPFH mutants[5–15]. How these mutations continue to express HbF in the adult and whether the structural anomalies of the $^G\gamma^A\gamma$ HPFH mutations affect the normal mechanism that controls haemoglobin switching, remain unknown.

In the present study, erythroid progenitors (BFU-E) were cultured in the conditions described in Fig. 1 legend. Cells were grown in semisolid media and mature erythroid clones collected after 15–17 days in culture, incubated with ^3H-leucine and used to measure the incorporation of radioactivity into the globin chains as described previously[16,17]. Media in control cultures contained fetal calf serum (FCS). In the experimental cultures, FCS was substituted by a preparation of fetal sheep serum (FSS) which potently induced HbF to HbA switching in cultures of neonatal BFU-E and BFU-E from persons with an acquired HbF elevation.

As shown in Fig. 1, cultures of cells from two $^G\gamma^A\gamma$ HPFH heterozygotes (having a mutation that deletes part of the $^A\gamma$ to δ flanking region, the δ and β genes and several kilobases 3′ of the β gene) produced 25–40% HbF in the control FCS cultures. In FSS cultures, however, HbF synthesis ranged from barely detectable to a maximum of 2%. Cells from two other $^G\gamma^A\gamma$ HPFH carriers, in whom the molecular lesion has not been defined, showed a similar degree of abrogation of HbF synthesis in FSS cultures. To test the response of non-deletion HPFH variants, we cultured erythroid cells from a heterozygote for the Greek $^A\gamma$ HPFH and a heterozygote for the African $^G\gamma$ HPFH. These cells produced 20–35% HbF in FCS cultures, but barely measurable amounts in FSS cultures (Fig. 1).

Several experiments were done to determine the reason for the inhibition of HPFH expression in FSS cultures. It is known that γ and β globins are produced asynchronously during erythroblast maturation, γ globin and γ mRNA accumulating in immature erythroblasts, with β-globin expression increasing in mature erythroblasts[18,19]. To test whether FSS decreased HbF through this maturational mechanism, deletion HPFH BFU-E were cultured and immature clones (composed of proerythroblasts and basophilic erythroblasts) or mature clones (composed of polychromatophilic and orthochromatic erythroblasts) were collected separately and used to assess globin biosynthesis. We found that the immature HPFH erythroblasts had the low γ-globin synthesis characteristic of FSS cultures, a result eliminating the possibility of maturational modulation and suggesting

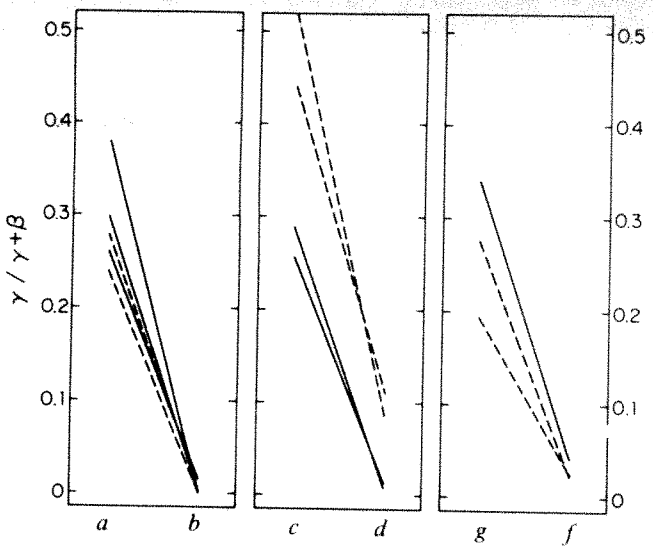


Fig. 1 Globin chain biosynthesis (expressed as $\gamma/\gamma+\beta$ ratio) in cultures of erythroid cells from humans heterozygous for hereditary persistence of fetal haemoglobin. *a*, Cultures performed in FCS. *b*, Cultures performed with FSS containing haemoglobin switching activity. Each line represents the results of a separate experiment. Left panel: results with cells of two $^{G}\gamma^{A}\gamma$ HPFH heterozygotes who, by globin gene restriction endonuclease mapping, were found to carry the Ghana type deletion HPFH mutation[11,15]. Middle panel: results from cultures of two $^{G}\gamma^{A}\gamma$ HPFH heterozygotes whose mutation has not been molecularly characterized. Right panel: findings in two non-deletion mutations, $^{G}\gamma$ HPFH (—) and Greek $^{A}\gamma$ HPFH (---). Note in all panels the striking reduction of γ-globin synthesis in *b*. In addition to the striking differences in $\gamma/\gamma+\beta$ ratios, the α/non-α ratios differed between FCS and FSS cultures (being 10–20% higher in the latter). This is expected, as turning off the γ-chain, synthesis in the mutant HPFH chromosome will result in α/non-α chain imbalance.
Methods: Peripheral blood was used as a source of erythroid progenitors. After Hypaque–Ficoll centrifugation interphase cells were collected, washed and adhered to plastic surfaces to remove the majority of adherent cells. Non-adherent cells were inoculated at appropriate concentrations ($1–3\times10^{5}$ ml^{-1}) in methylcellulose in the presence of erythropoietin, T-cell-derived conditioned medium and either FCS or a FSS preparation known to contain Hb switching activity. In these conditions erythroid progenitors (burst forming units, BFU-E) produce large, multi-lobed erythroid colonies (erythroid bursts) that mature in culture during days 13–20. Mature erythroid bursts were lifted from the plates, pooled according to experimental category and used to measure globin chain synthesis.

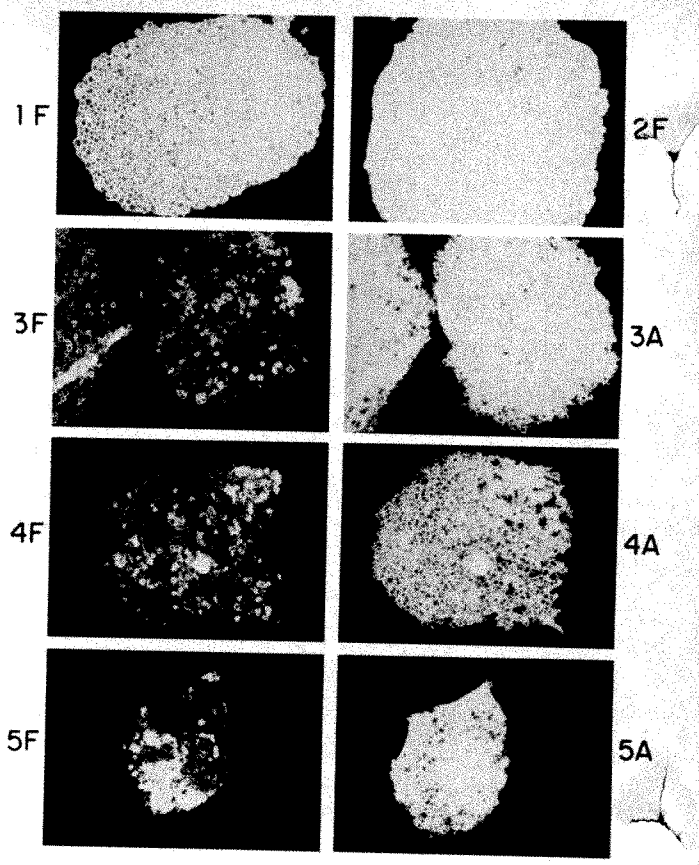


Fig. 2 Immunofluorescent labelling of HPFH clones. Mature clones were lifted from the cultures, fixed and labelled with an anti-γ chain monoclonal antibody and rabbit anti-mouse F(ab)$_2$-fluorescein isothiocyanate (FITC) and counter-labelled with an anti-β globin chain monoclonal antibody conjugated to rhodamine. This double-labelling approach allows detection of both γ and β globins in each cell of a clone. 1F and 2F show two FCS-grown clones labelled with the anti-γ globin–FITC. Note the strong labelling of all the cells. 3, 4 and 5 are FSS-grown clones labelled with anti-γ FITC (3F, 4F, 5F) and anti-β rhodamine (3A, 4A, 5A). Note that in 3F, 4F and 5F the majority of cells either fail to be labelled by the anti-γ-chain antibody or are labelled very faintly, showing the reduced total accumulation of HbF in the cells of FSS-grown clones.

that the FSS-grown immature HPFH erythroblasts already have been programmed to produce low amounts of HbF. To test whether our findings reflected an absolute reduction of γ-globin chain production, we performed experiments using fluorescent anti-globin chain monoclonal antibodies. A double-labelling procedure (described in Fig. 2 legend) was used and over 50 clones (per experiment) were analysed for γ- and β-globin expression. Clones grown in FCS displayed the pancellular expression of HbF characteristic of HPFH mutations (Fig. 2) whereas in most FSS-grown HPFH clones, γ globin was barely detectable. In other experiments, some cells from FSS-grown clones expressed HbF (Fig. 2). These results showed that culture in FSS results in a marked reduction of γ-chain expression in the HPFH erythroblasts.

To test directly whether HbF was turned off through an interaction between HPFH cells and the switching activity in FSS cultures, experiments were performed using split clones. Deletion HPFH BFU-E were cultured in either FCS or FSS. When the clones had reached a size of about 50 cells (five to six progenitor cell divisions), each clone was divided into two almost equal parts, one part being transferred to a plate containing FCS, the other part to a plate containig FSS. The clones were left to grow and mature (five to six cell divisions) and then used for globin analysis. As shown in Table 1, there are striking differences in HbF production between the parts of a clone transferred to FSS or to FCS, showing directly that the progeny of the same HPFH progenitor can produce high or low HbF depending on the environment. Of special significance are the low HbF levels observed in the FSS to FCS transfers (Table 1); they show that the expression of the HPFH mutation can be altered even when the cells forming a clone are exposed to the inductive environment only during the initial stages of their *in vitro* differentiation.

The present findings in HPFH cultures are identical to findings in normal and haemoglobinopathic cell cultures reported by us previously[2–4]. The fact that the HPFH cells return to the normal pattern for adult cells (that is, turning off HbF expression) when cultured in FSS suggests that the HPFH mutations can be modulated by exogenous factors. Previously, the phenotypic consequences of HPFH mutations have been interpreted to mean that in the deletion mutations, a *cis*-acting regulatory element which usually turns off the γ genes is deleted, while the non-deletion HPFH are mutations of *cis*-acting elements that regulate the individual $^{G}\gamma$ or $^{A}\gamma$ genes. The results presented here are compatible with an alternative postulate: in HPFH, the response of the mutant globin genomic regions to normal

Table 1 Synthesis of fetal haemoglobin in divided HPFH clones

Clone no.	Initial growth in	$\gamma/\gamma+\beta$ in part of clone grown in FCS	FSS
1	FCS	0.26	0.01
2	FCS	0.35	0.03
3	FCS	0.22	0.01
4	FCS	0.33	0.02
5	FCS	0.24	0.03
6	FCS	0.59	0.02
7	FCS	0.33	0.06
8	FSS	0.18	0.02
9	FSS	0.13	0.01
10	FSS	0.12	0.02
11	FSS	0.07	0.01
12	FSS	0.15	0.03
13	FSS	0.19	0.02
14	FSS	0.15	0.01

Clones were grown in FCS or FSS. At the 30–50-cell stage the clone was divided into two parts by *in situ* micromanipulation. Using an ultrathin pipette, one part of the clone was lifted and implanted in a cell-free methylcellulose plate containing FCS; the other part of the clone was lifted and implanted in a cell-free methylcellulose plate containing FSS. The transferred parts of the clones were left to grow and mature. At maturation the two parts were of similar sizes (as shown by surface counting). The mature clones were plucked out of the plates, and each part was incubated with ^{3}H-leucine separately and used for globin chain isoelectric focusing to measure the $\gamma/\gamma+\beta$ globin chain synthesis ratio.

trans-acting regulatory elements that control haemoglobin switching may be altered, so that higher levels of *trans*-acting regulatory elements are required to turn off the expression of HbF in the mutant adult cells. The differences in the degree of inhibition of HbF noted in the split clone transfers (Table 1) and in the studies of single clones by immunofluorescence support this postulate. The hypothesis can also explain the paradox that HbF can be turned off *in vitro* in mutants that express this haemoglobin *in vivo* in conditions of normal erythropoiesis.

Previous work has shown that adult erythroid progenitors have an inherent potential to form progeny which express fetal haemoglobin[20]. This HbF potential is presumably lost during downstream differentiation through interactions between progenitors and the inductive environment of adult haematopioesis[2,3,20]. In HPFH mutations, the structural anomalies in the globin genomic region are expected to augment the HbF potential of erythroid progenitors. In the conditions of haematopoietic differentiation that prevail in the marrow of HPFH heterozygotes, the normal level of the inductive environment is not enough to modulate the augmented HbF potential of HPFH progenitors, and terminal cells expressing HbF are formed. In FSS cultures, haemoglobin switching activity is present in excess throughout erythroid cell development, allowing a degree of cell–environment interaction that can turn off the augmented HbF potential of HPFH progenitors; as a result, erythroblasts lacking HbF are produced.

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Contribution of human $V_{\kappa II}$ germ-line genes to light-chain diversity

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The genetic basis of the antibody repertoire—estimated to exceed 10^6 different immunoglobulin molecules—is a major unanswered problem[1,2]. The number of germ-line V_κ genes in the mouse genome is probably several hundred[3,4] while the corresponding number for three out of four human V_κ subgroups ($V_{\kappa I}$, $V_{\kappa III}$ and $V_{\kappa IV}$) is probably altogether only 15–20 (ref. 5). The κII proteins differ significantly in sequence from the other κ-chain proteins[6]. To determine the contribution of $V_{\kappa II}$ genes to κ-chain diversity, we searched for a human lymphoid cell line which produces a κII chain and report here for the first time the sequence of a $V_{\kappa II}$ gene. According to blot hybridizations with this V_κ gene as a probe, subgroup II contributes about half as many genes to the V_κ gene repertoire as are detected by a $V_{\kappa I}$ probe. Therefore the repertoire is rather small which implies that somatic mutations[7–9] or other mechanisms must play an important role in the generation of light-chain diversity in humans.

Subgroups I–III (refs 10, 11), and IV (ref. 12) were defined on the basis of the amino acid sequences of κ-type Bence–Jones and myeloma proteins. All human κ chains now known fit into this classification[6]. Recently, it has become clear that both a number of germ-line V_κ genes and somatic processes, including somatic point mutations, contribute to the sequence variation among light chains[1,2]. Recent advances in molecular cloning have made it possible to estimate the relative contribution of germ-line V_κ genes to the light-chain diversity. $V_{\kappa I}$ and $V_{\kappa III}$ probes hybridized not only with each other but also detected, in blot experiments with digests of total human DNA, essentially the same bands corresponding probably to the same 15–20 V_κ genes[5]. Since subgroup I and III proteins are more closely related to subgroup IV proteins than to one another, it was suggested[5] that these 15–20 genes may also contain the $V_{\kappa IV}$ genes.

The development of subgroup specific antisera[13] made it possible to assign κ chains to the subgroups without determining the amino acid sequences. Using such antisera in immunodiffusion assays with supernatants from various cell lines we found that the κ chain excreted by the human lymphoblastoid cell line GM 607 belongs to subgroup II. The cell line GM 607 which had been used in a number of studies (see, for example, ref. 14) contains one rearranged and one germ-line C_κ gene. The rearranged V_κ–C_κ region was cloned (Fig. 1) and the V_κ gene region sequenced (Fig. 2). The amino acid sequence which was obtained from the DNA sequence confirms the serological assignment of the GM 607 κ chain to subgroup II. Codons 97–108 are derived from the gene segment J1. The assignment of the sequence to subgroup II is unequivocal since all subgroup specific amino acids were found in the characteristic positions[6] and there is a 98% homology to the published sequence of the

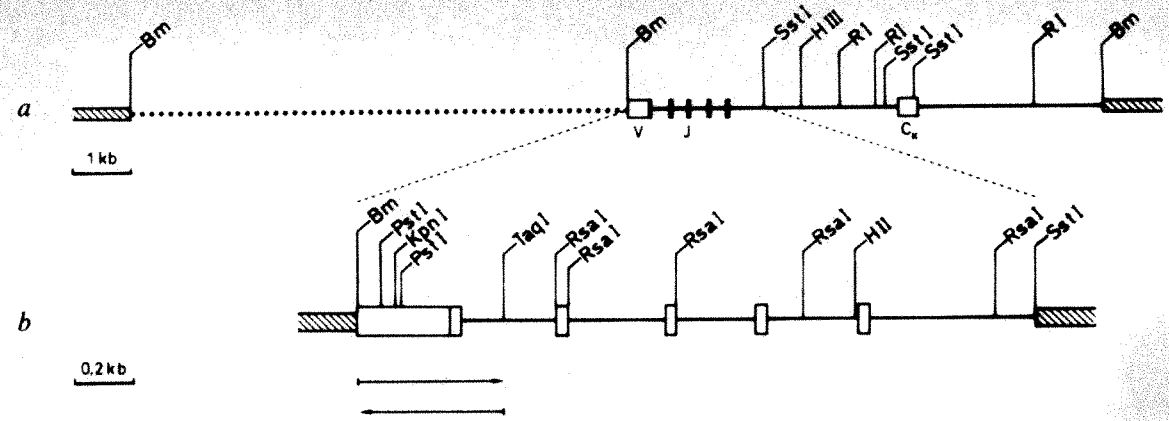


Fig. 1 Restriction map and sequencing strategy of a V_κ gene cloned from DNA of the lymphoblastoid cell line GM 607. The cell line which had been derived from a clinically unaffected person was obtained from the Human Genetic Mutant Cell Repository (Camden, New Jersey). A *Bam*HI digest of GM 607 DNA was electrophoretically separated and the 8-kb region cloned in Charon 30 (ref. 20). This is the region which hybridized with a cloned 2.6-kb *Eco*RI fragment from the human C_κ region[21]. The C_κ clone was derived from a 10.6-kb *Bam*HI clone which in turn had been isolated from human placenta DNA using a mouse J_κ region clone (1.56-kb *Hin*dIII–*Hin*dII; ref. 22). From the GM 607 clone (*a*) which contains an unrelated DNA fragment (.....) in addition to the κ gene, the subclone (*b*) was prepared in a pBR322 derivative which contains a *Sst*I site instead of the *Hin*dIII site. For sequencing, the *Bam*HI–*Taq*I fragment was cloned in M13mp8 and 9 (ref. 23). Bm, *Bam*HI; HII, *Hin*dII; HIII, *Hin*dIII; RI, *Eco*RI.

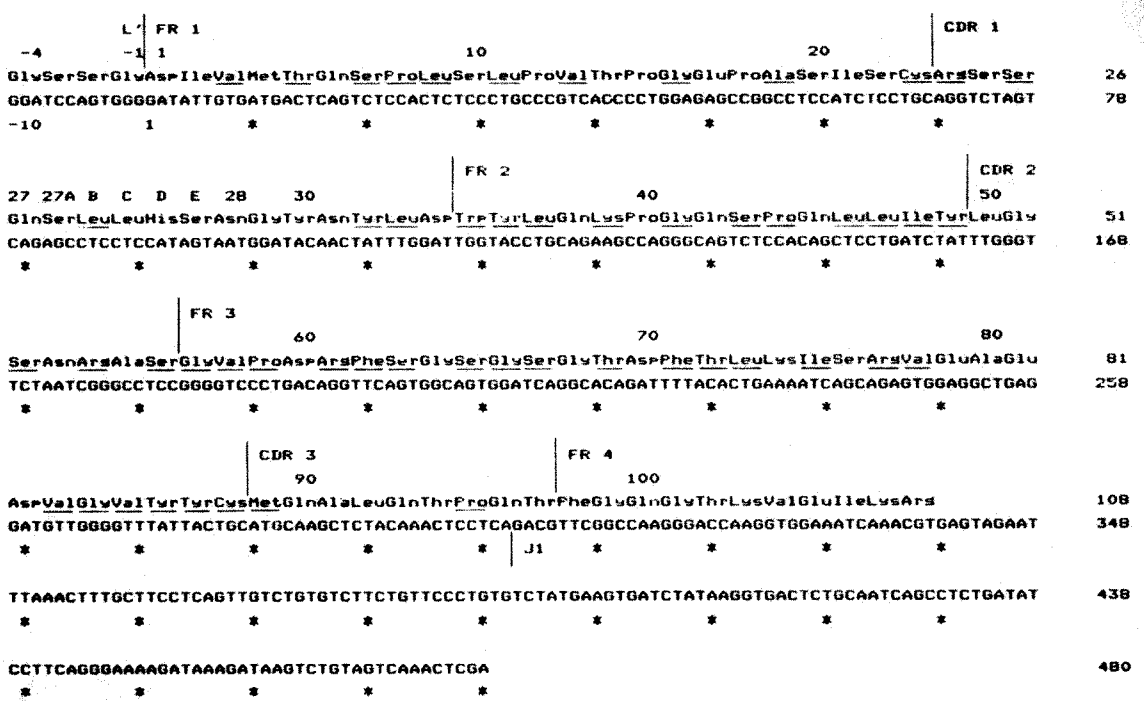


Fig. 2 Sequence of the V_κ–J_κ gene region of the rearranged allele of GM 607. The M13mp8 and mp9 subclones (Fig. 1) were sequenced by the chain terminator method[24] using 1-m long gels (ref. 25 and H. R. Jaenichen *et al.*, manuscript in preparation). Numbering of codons/amino acids is according to Kabat *et al.*[6]. $V_{\kappa II}$ specific amino acids are underlined. The J1 and intron sequence is identical to the sequence of Hieter *et al.*[21] except for a GTG instead of a GTTG and a C instead of a T (positions 401–403 and 436, respectively, in our numbering). Regions: L', leader; FR, framework; CDR, complementarity determining.

Bence–Jones protein TEW[15]; there is 93% homology, if gaps are counted as one mismatch each and amide differences are included. Since our clone does not extend as far in the 5′ direction as the region in which intron and leader sequences would be located, the assignment of codons −1 to −4 to amino acids of the leader remains tentative.

The sequence of the V_κ gene of GM 607 shares 64% homology with the published sequence of the $V_{\kappa I}$ gene HK 102 (ref. 16) which has been confirmed and extended in our laboratory (H. R. Jaenichen *et al.*, manuscript in preparation). In this sequence comparison the 15 base pairs (bp) from position 82 to 96 which are not present in the $V_{\kappa I}$ gene are counted as one mismatch. The homologies between the complementarity determining regions of the $V_{\kappa I}$ and $V_{\kappa II}$ genes were found to be low: CDR1, 55%; CDR2, 33%; CDR3, 29%. Although the framework regions of the two genes show fairly high homologies (FR1, 62%; FR2, 80%; FR3, 76%) the degree of cross-hybridization was very low. In dot blot[17] experiments (Fig. 3) 3% of cross-hybridization were found under stringent and 4–5% under non-stringent conditions. In Southern blot hybridizations[18] cross-hybridization between the subgroup I and II genes was barely detectable.

To estimate the approximate number of germ-line $V_{\kappa II}$ genes restriction nuclease digests of total human DNA were hybridized

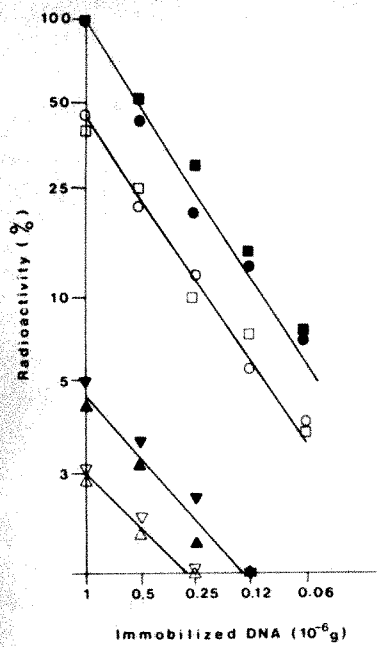


Fig. 3 Cross-hybridization between V_κ genes of subgroups I and II. The DNAs used were derived from the GM 607 subclone in M13mp9 (Fig. 1) ($V_{\kappa II}$) and from a $V_{\kappa I}$ clone in M13 phage. The inserts comprise 492 and 550 bp, respectively, the latter one being identical to the *Pst*I fragment of HK 102 (ref. 16; H. R. Jaenichen *et al.*, manuscript in preparation). The inserts were self-ligated to sizes of at least 5 kb. Approximately 0.06 to 1 μg DNA each of the DNAs were applied to nitrocellulose paper and immobilized[17]. The same DNAs were nick-translated to about equal specific radioactivities with [α-^{32}P]dATP and [α-^{32}P]dCTP[26]. Self- and cross-hybridizations were carried out in 6×SSC at 68 °C; washing was with 6×SSC or down to 0.1×SSC (non-stringent and stringent, respectively). Individual dots were cut out (1 cm^2 each), counted, and the values plotted (after subtraction of blanks); non-stringent self-hybridizations (~4,000 c.p.m.) were taken as 100%. Self-hybridizations with $V_{\kappa I}$ DNA (○, ●) and $V_{\kappa II}$ DNA (□, ■), cross-hybridizations with $V_{\kappa I}$ (△, ▲) and $V_{\kappa II}$ (▽, ▼) DNAs as probes; open and closed symbols represent stringent and non-stringent conditions.

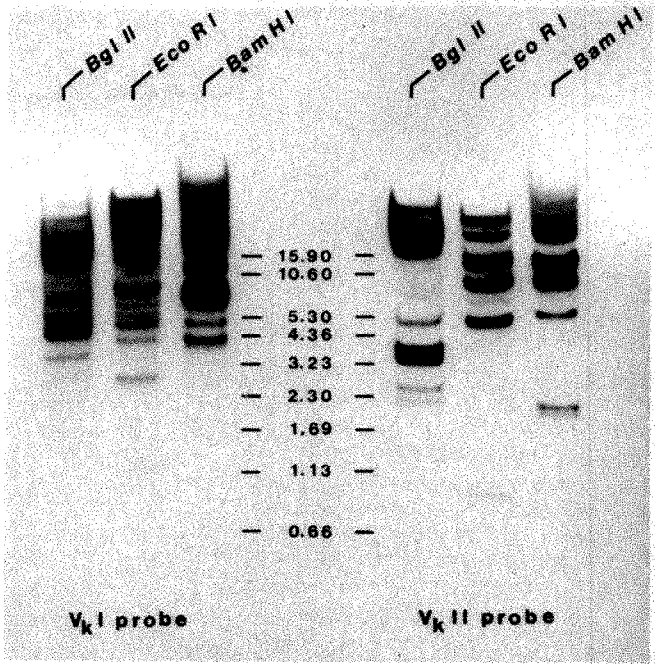


Fig. 4 Hybridization of restriction nuclease digests of total human DNA with $V_{\kappa I}$ and $V_{\kappa II}$ probes. 15 μg each of *Bam*HI, *Eco*RI and *Bgl*II digests of human placenta DNA were separated electrophoretically on 6 mm thick 1% agarose gels in a buffer (similar to ref. 27), 40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, *p*H 7.4. After blotting[18] hybridization with the nick-translated probes (Fig. 3) was in 3×SSC at 68 °C and washing in 2×SSC. Autoradiography was without intensifying screens.

under various conditions with the $V_{\kappa II}$ and, for comparison, the $V_{\kappa I}$ probes (Fig. 4). With the $V_{\kappa I}$ probe we obtained band rns very similar to those of Bentley and Rabbitts[5] who ɔreted them to represent 15–20 V_κ genes. Such an interpretation is possible since the authors found only little polymorphism of restriction sites in the V_κ locus; they also took into account, as far as possible, the different intensities of the bands in the blot hybridization experiments. On the same basis we estimate that the $V_{\kappa II}$ subgroup contains about half as many genes as are detected with the $V_{\kappa I}$ probe. The strong bands of the $V_{\kappa II}$ patterns cannot represent $V_{\kappa I}$ genes because cross-hybridization between the two DNA probes is negligible.

Any figure for the number of different genes in the human V_κ locus which is based on the counting of bands in an electrophoretic separation can be taken only as an estimate. The sequence divergence within the subgroups could be rather high so that a subgroup-specific probe would detect extreme variants only as weak bands. The degree of cross-hybridization between the subgroups, on the other hand, is difficult to predict on the basis of amino acid or even nucleic acid sequences; this may be a source of both possible over- and underestimates of gene numbers. The presence of very homologous genes probably arising from recent gene duplication(s) in the V_κ locus, means that the actual number of V_κ genes detected by a subgroup I probe may be somewhat higher[19] than originally proposed[5]. When several hybridization probes are available for each of the four subgroups it will be possible to make more exact estimates. The number of V_κ genes will be known definitely only after the whole V_κ locus has been cloned. Since only sequencing can distinguish between potentially functional genes and pseudogenes, the exact determination of the functional V_κ gene repertoire is a long-term project. Present estimates, however, clearly indicate that in humans the V_κ gene repertoire is relatively small and that other mechanisms, for example, somatic mutation, must contribute significantly to the generation of the V_κ-sequence diversity.

After submission of the present report, a paper was published[28] describing the sequence and hybridization properties of a $V_{\kappa III}$ cDNA. The results are interpreted to be in agreement with Bentley and Rabbitts[5] and 50 or less germ-line V_κ genes are proposed.

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Isolation of a cDNA clone coding for an SB β-chain

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Class II antigens of the major histocompatibility complex (MHC) consist of a family of closely related cell surface-expressed glycoproteins. These antigens, which are genetically polymorphic, control important aspects of the immune response[1]. At least three types of human class II antigens, namely, DR, DC and SB (refs 2–4), have been identified. All class II antigens are heterodimers composed of one α- and one β-chain. The genes for both types of subunits are encompassed within the MHC[5,6]. The general features of the DC and DR antigens have recently been elucidated[7–15]. Much less is known, however, about the SB molecules[16]. Here we describe the isolation of a cDNA clone as well as a genomic clone encoding a β-chain whose amino acid sequence is compatible with the partial amino-terminal sequence of SB β-chains[16].

Recent studies have shown that part of the 3′-untranslated regions of DR and DC cDNA clones can be used as locus-specific probes in hybridization experiments[17]. This information prompted us to isolate non-DR, non-DC locus-derived cDNA clones from a previously described library[18]. Colony hybridizations were carried out separately using probes corresponding to the translated and the 3′-untranslated portions of DR and DC β cDNA clones, respectively[12,19]. One cDNA clone, pII-β-7, hybridized to the probes corresponding to the translated portions but did not hybridize to the 3′-untranslated probes. Therefore, pII-β-7 was characterized by restriction mapping and nucleotide sequence analysis. Figure 1 summarizes the results and displays the amino acid sequence predicted from the nucleotide sequence; the data demonstrate that pII-β-7 is truncated in the 5′ region as only three of the four cysteine codons, characteristic for all β-chains sequenced so far[9,10,12,14] are present in the clone (compare Fig. 2).

To deduce the amino-terminal sequence of a β-chain of the same type as that encoded by pII-β-7, we isolated a genomic clone from a DR/Dw 4/4 cosmid library (B. Servenius *et al.*, in preparation) with this cDNA clone as the probe. This cosmid clone, cosII-412, contains a β gene that is related to pII-β-7 according to the following criteria: the 3′-untranslated sequences of the two clones show 90% homology (data not shown). No homology whatsoever is found between the 3′-untranslated portions of these clones and clones encoding DR and DC β-chains. The exon encoding the first domain of cosII-412 is highly homologous to the portion of pII-β-7 available for comparison. Also, the amino acid sequences predicted from the two clones are homologous (Fig. 1*b*). The six nucleotide substitutions between the sequenced portions of cosII-412 and pII-β-7 (derived from a DR 3/w6 library) are not unexpected as the polymorphism of the β-chains is almost exclusively localized in the first domain[10].

pII-β-7 and cosII-412 encode β-chains that are distinct from, but homologous to, DR and DC β-chains (Fig. 2). To obtain maximal homology with the latter sequences, a gap of two residues had to be introduced into the amino acid sequence predicted from cosII-412. Accordingly, the acceptor site of the first splice junction in cosII-412 resides in the same codon (+5) as in DC β (ref. 20) and DR β genes (D. Larhammar *et al.*, in preparation), assuming that the same number of amino acids are encoded by the signal sequence exons. The extracellular portions of the β-chains corresponding to pII-β-7 and cosII-412

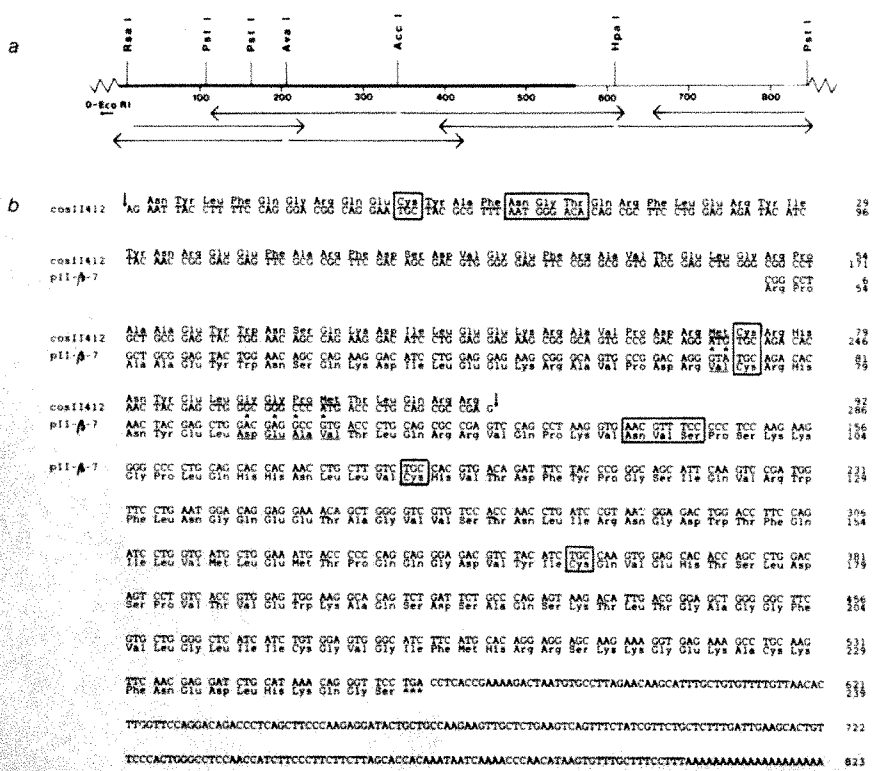


Fig. 1 *a*, Restriction map of the pII-β-7 cDNA insert, with the translated portion indicated by a thick line. The shortest distance from the cloning site (*Pst*I) to the *Eco*RI site at position 0 in pBR322 is indicated. The sequencing strategy is shown by arrows. *b*, The nucleotide and predicted amino acid sequences of the cDNA insert of pII-β-7 and of the first domain exon of cosII-412. Asterisks denote nucleotide substitutions between the two sequences. Amino acid replacements are underlined. The nucleotide sequence was determined according to the procedure of Maxam and Gilbert[22]. Cysteine residues, which by homology to other class II antigen β-chains are disulphide-linked, are boxed, as are two putative attachment sites for *N*-linked oligosaccharides at Asn 19 and Asn 98. The first cysteine residue is numbered 15 in accordance with DR and DC β sequences. cosII-412 was isolated from a genomic library consisting of DNA from an Dw/DR 4/4 individual partially digested with *Mbo*I and inserted into the cosmid vector pHEP (ref. 23). pII-β-7 was used as the probe in colony hybridization. cosII-412, which hybridized strongly to the probe, contained the first domain exon in a 7.3-kb *Hin*dIII fragment, which was subcloned into pUC9 and used for nucleotide sequencing.

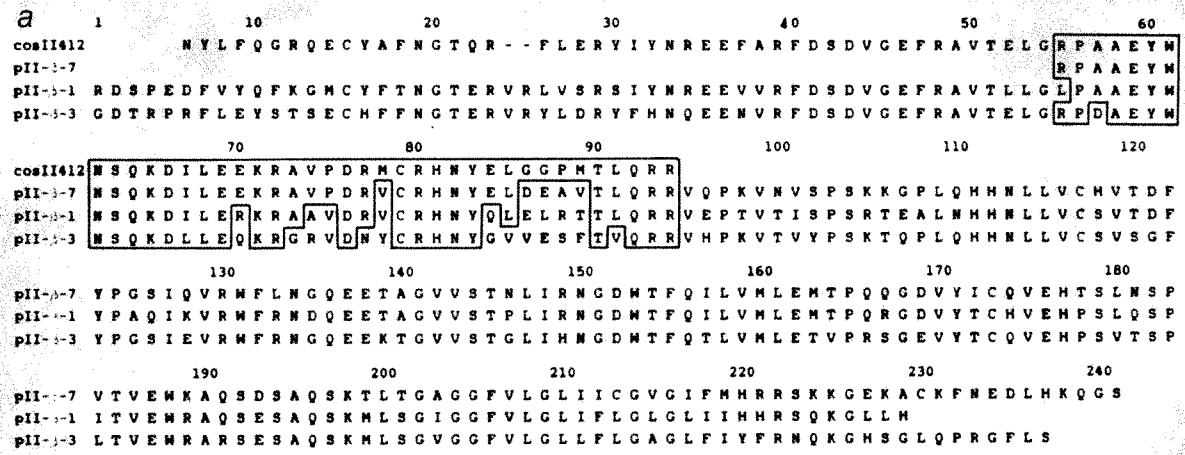


Fig. 2 *a*, Comparison of the amino acid sequences predicted from pII-β-7 and cosII-412 with the corresponding sequences of pII-β-1 (DC)[11] and pII-β-3 (DR)[19]. A gap of two residues was introduced into the sequence predicted from cosII-412 to maximize the homology. Amino acid residues homologous to the cosII-412 β-chain in the portion where this sequence overlaps the pII-β-7 β-chain sequence, are boxed. *b*, Comparison of the partial amino-terminal sequence of an SB β-chain[16] with the predicted amino acid sequences of cosII-412, cosII-102 (ref. 20), pII-β-1 (ref. 12), pII-β-2 (ref. 10), pII-β-3 and -4 (ref. 19) and HLA-DRβ1 (ref. 14). Tyrosine and phenylalanine residues are enclosed in open or shaded boxes. Shaded residues coincide with the tyrosines and phenylalanines present in the SB β sequence.

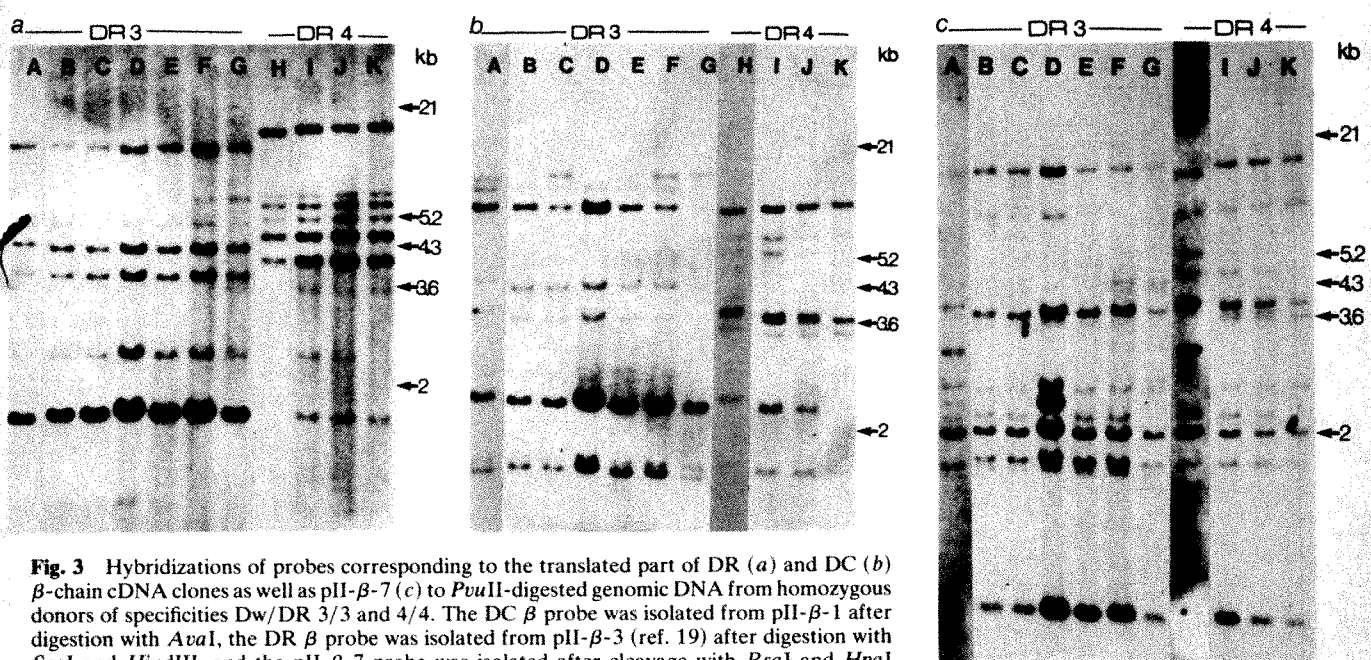


Fig. 3 Hybridizations of probes corresponding to the translated part of DR (*a*) and DC (*b*) β-chain cDNA clones as well as pII-β-7 (*c*) to *Pvu*II-digested genomic DNA from homozygous donors of specificities Dw/DR 3/3 and 4/4. The DC β probe was isolated from pII-β-1 after digestion with *Ava*I, the DR β probe was isolated from pII-β-3 (ref. 19) after digestion with *Sac*I and *Hin*dIII, and the pII-β-7 probe was isolated after cleavage with *Rsa*I and *Hpa*I (Fig. 1). All probes are derived from the translated regions of the three β cDNA clones. The probes were nick-translated (ref. 24) to a specific activity of $5–10 \times 10^7$ c.p.m. μg^{-1}. DNA was isolated from the cells as described previously[25]. DNA samples (10 μg) were digested to completion with *Pvu*II (New England Biolabs) and run on 0.7% agarose gels in 89 mM Tris-borate buffer, *p*H 8.3 containing 2.6 mM EDTA for 600 volt-hours and transferred to nitrocellulose filters[26]. The filters were prehybridized for 3–6 h at 42 °C in 50% formamide, 5 × SSPE buffer (SSPE = 10 mM sodium phosphate *p*H 7.0, containing 1 mM EDTA, 180 mM NaCl), 5 × Denhardt's solution[27], 0.02% of salmon sperm DNA and 0.2% SDS. Radioactively labelled cDNA fragments were added ($1–2 \times 10^6$ c.p.m. ml^{-1}) in a solution of 50% formamide, 5 × SSPE buffer, 1 × Denhardt's solution, 0.02% salmon sperm DNA, 0.2% SDS and 10% dextran sulphate. After hybridization at 42 °C for 16–24 h, the filters were washed twice in 2 × SSPE buffer, 0.2% SDS for 5 min at room temperature and twice in 0.2 × SSPE buffer, 0.2% SDS for 20 min at 55 °C. The filters were then autoradiographed with intensifying screens at −70 °C.

display approximately 67 and 71% homology to DR and DC β-chain, respectively. The latter two types of β-chains are approximately 66% homologous with each other (Fig. 2*a*). Thus, pII-β-7 and cosII-412 encode a new type of class II antigen β-chain. This is also evident from the predicted structure of the cytoplasmic portion of the pII-β-7 β-chain; it consists of 22 amino acids in contrast to the cytoplasmic portions of DR[14] and DC[10,12,20] β-chains, which are composed of 16 and 10 residues, respectively.

A partial amino-terminal sequence of an SB β-chain has been determined, locating the positions of five tyrosine and two phenylalanine residues[16]. Except for one tyrosine, all these residues coincided with tyrosines and phenylalanines in the amino acid sequence predicted from cosII-412 (Fig. 2*b*). Moreover, no additional residues of these types were found in the sequence. In contrast, of the seven residues identified in the SB β-chain, only two (or three) tyrosines coincided with tyrosines in DC β-chains and only one phenylalanine with the corresponding residue in DR β-chains. One DR β-chain sequence also displayed one of the tyrosines found in the SB β-chain. The DC and DR β-chains also contained additional tyrosine and phenylalanine residues at the amino terminus. We suggest, therefore, that cosII-412 and pII-β-7 encode SB β-chains (or chains closely related to SB β).

The SB locus is located at some distance towards the centromere from the DR and DC loci, which are tightly linked[4]. To study the degree of linkage between the gene corresponding to pII-β-7 and the DR locus, we used pII-β-7 as a hybridization probe to genomic DNA of HLA D/DR homozygous individuals. For comparison, hybridizations were also carried out with DR and DC β-chain probes. The DNA samples (seven of which were typed to be Dw/DR 3/3 and four to be Dw/DR 4/4) were digested with *Pvu*II. The experimental conditions chosen for the hybridizations were such that no, or only slight, cross-hybridizations occurred with the three probes. Figure 3 shows that the banding patterns obtained were distinct and different. The probe corresponding to the translated portion of a DR β-chain clone gave rise to virtually identical patterns for all the DR 3/3 homozygous samples (Fig. 3*a*). It should be noted that the faint band of ~6 kilobases (kb) was present in all samples and that the figures are composites from several autoradiograms, thus making perfect alignments of the samples difficult to achieve. The DR 4/4 samples gave a pattern distinct from that of the DR 3/3 samples. However, apart from a weakly hybridizing band of 2.2 kb which was absent from one sample (Fig. 3*a*, lane K) all DR 4/4 homozygous samples appeared identical.

The DC β probe also gave rise to hybridization patterns that conformed well to the typed DR specificity of the sample. Thus, all seven DR 3/3 homozygous samples appeared identical, apart from one sample lacking a 9.2-kb band (Fig. 3*b*, lane D). This result agrees with the observation that the hybridization patterns of DR 4/4 homozygous samples, although different from those of the DR 3/3 samples, were also identical to each other. It is obvious that the 2.2-kb band, absent from lane K of Fig. 3*b*, was the result of cross-hybridization of the DC and DR β-chain probes to the same genomic sequence (compare Fig. 3*a*, lane K).

The hybridization patterns obtained with pII-β-7 as the probe were quite similar for the DR 3/3 and DR 4/4 samples (Fig. 3*c*). In addition, the variability between identical DR samples was of the same magnitude as between the DR 3/3 and DR 4/4 samples. Thus, the hybridization patterns did not correlate well with the DR specificity of the samples. This indicates that the locus corresponding to pII-β-7 and cosII-412 is located at some distance from the DR locus. This finding is perfectly compatible with the structural similarity between the β-chain encoded by cosII-412 and an isolated SB β-chain discussed above. We conclude, therefore, that pII-β-7 and cosII-412 code for SB β-chains (or chains closely related to SB β-chains).

After submission of this manuscript, Roux-Dosseto *et al.* reported the identification and sequence of a cDNA clone suggested to correspond to an SB β-chain[21]. The β-chain predicted from this clone is highly homologous to that deduced from pII-β-7, except in the cytoplasmic portion, which only consists of 12 residues. Whether the clone described by Roux-Dosseto *et al.*[21] is derived from the same gene as the clones described here remains to be investigated.

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Immortalization of monkey epithelial cells by specific fragments of Epstein–Barr virus DNA

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Epstein–Barr virus (EBV) is unique among the DNA tumour viruses by virtue of its association with two human malignancies, Burkitt's lymphoma and nasopharyngeal carcinoma (NPC), the former a tumour of B lymphocytes and the latter encompassing low-differentiated epithelial cells of the nasopharynx[1–4]. A viral gene product has not been definitively linked to these malignant diseases, although an EBV nuclear antigen(s) (EBNA) seems to be ubiquitous in EBV-infected cells; indeed, the detection of EBNA by immunofluorescence is often taken as an indication of the presence of the viral genome[5,6]. As part of a study to investigate which part of the EBV genome is responsible for transformation and whether the same mechanism of cellular transformation is involved in the case of B lymphocytes and epithelial cells, we have tried to establish whether a detectable cellular alteration(s) can be induced in primate epithelial cells by the presence of a specific region of the EBV genome. We report here that it can—the result is immortalization of the cells.

A hybrid cosmid library was made that encompasses the viral genome within six fragments (see Fig. 1), using partially cleaved *Bam*HI fragments from B95-8 virion DNA cloned into the cosmid pHC79 (ref. 7). These recombinants, together with virion and episomal[8] EBV DNAs, and suitable controls, were used to transfect a mixture of cells derived from African green monkey (*Cercopithecus aethiops*) kidneys (AGMK cells). After

~3 months in culture, very small, densely staining cell populations were observed in several of the experiments but the two dishes that contained cells transfected with DNA from cosmid clones designated p13/p33 and p31 were readily distinguishable from the others by the quantity and size of 'foci' in them. Details of the transfection experiments, and data derived from them, are given in Fig. 2. The results illustrated in Fig. 2 were confirmed by examination of unstained dishes under the light microscope.

Individual foci were selected from AGMK cells transfected with p13/p33 and p31 DNAs, as well as from dishes containing cells transfected with either calf thymus (control) or clone p65 DNAs. Foci were transferred manually to fresh dishes (50 mm) and fed weekly. Whereas none of the small foci from the calf thymus or p65 experiments grew, nine cell lines were established from foci (out of the 10 selected from each experiment) derived from the p13/p33 or the p31 dishes. Thus, only AGMK cells exposed to EBV DNA fragments from a specific region of the viral genome could be propagated in culture. Several of the cell lines have been studied further. Five of them subdivided at a 1:10 dilution weekly have been kept for more than a year in culture (corresponding to at least 200 generations) with no apparent change in morphology or growth rate, as determined by regular examination by light microscopy. By morphological and histological examination the cells have been shown to be epithelial in type (see Fig. 3*a–c*). Cells from the 13/33 f4 and the 31 f4 experiments, screened by immunofluorescence, show the presence of intermediate filaments typical of epithelial cells[9,10] (see Fig. 3*d, e*). Whereas microscopic examination of the original AGMK cells revealed cells characteristic of elongated and cuboidal epithelium, and of fibroblasts, the immortalized cells exhibited only the simple cuboidal morphology (Fig. 3*b, c*). Thus, it seems that a subpopulation of monkey epithelial cells has been immortalized by transfection with the EBV DNA fragments.

All cell lines examined proved to be capable of growing for a limited time in low serum. Cell growth in either normal (5%) or low (0.5%) concentrations of fetal calf serum (FCS) was independent of serum concentration during the first month in culture, cells being subdivided at a 1:10 dilution weekly and cell counts determined. After 2 months, the growth rate in low serum slowed to about one-half that of the cells growing in normal serum. Qualitatively similar results were obtained when the cells were plated into semi-solid (agar) media, that is, initially the cells divided and small visible colonies, containing a number of greatly enlarged cells, were observed within 3–4 weeks. These colonies generally then ceased to undergo further division, but ›eing isolated and re-plated onto plastic, they resumed ion and grew normally. In additional experiments, cells ı early passages of a number of the lines, as well as control AGMK cells, were injected intraperitoneally into young nude mice (10^6 cells per animal); no tumours were apparent a year later. (Similar experiments using late passage cells are in progress.) These observations all indicate some dramatic alteration in the growth properties of the cells, effected by specific fragments of EBV DNA, that are not sufficient, however, to produce a malignant phenotype.

As a permanent change seemed to have been produced in the AGMK cells following transfection with either of two defined, partly overlapping fragments of the EBV genome, it remained to be determined whether the continued presence of the virus-derived DNA could be observed. Therefore, total chromosomal DNA from four of the established cell lines was applied directly to nitrocellulose filters and probed with either ^{32}P-labelled B95–8 virion DNA, or cloned fragments of EBV DNA, in order to detect viral DNA sequences. The presence of EBV-related sequences was observed in all four cell lines analysed, but not in the calf thymus DNA used as a control (data not shown). To examine further the nature of EBV DNA sequences in the cell lines, cellular DNA was cleaved with the *Bam*HI restriction enzyme and the resulting fragments were analysed by established methods[11]. Specific fragments that hybridized to viral DNA were observed (Fig. 4); the patterns

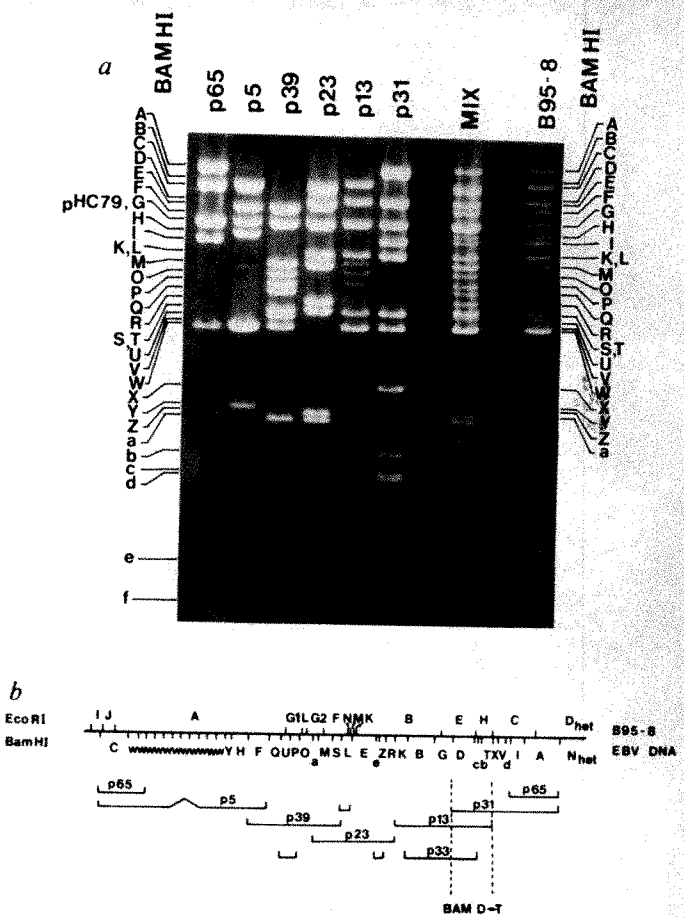


Fig. 1 ***a***, Cloned large fragments of EBV DNA. Virion EB DNA[22] (5 μg) from B95-8 marmoset cells was partially digested with *Bam*HI, and fractionated by neutral sucrose density gradient centrifugation to reduce contamination with small fragments. DNA fragments of ~40 kilobases (kb) were ligated to the *Bam*HI-cleaved cosmid pHC79 DNA (which had been dephosphorylated with alkaline phosphatase), and the resulting hybrid DNAs were cloned according to the procedure of Hohn[23]. Isolated clones were initially analysed by the 'dot-blot' method using individually the large *Eco*RI fragments A to G (ref. 24), radioactively labelled with ^{32}P according to Rigby *et al.*[25], as probes. DNAs from clones that yielded results consistent with the known physical map of EBV DNA were further analysed by electrophoresis on 0.8% agarose gels in Tris-acetate buffer in the presence of ethidium bromide and visualized with UV light, after treatment with *Bam*HI. A 'library' was selected, from among 780 clones analysed, which consisted of six clones designated p65, p5, p39, p23, p13 and p31, as shown. A mixture of the cloned DNAs (MIX) produced a pattern that was qualitatively indistinguishable from the viral (B95-8) DNA. The location of known fragments (A to e) and pHC79, which co-migrates with *Bam*HI G, are indicated; the presence of the latter was confirmed by hybridization against DNA from a clone of *Bam*HI G fragment (data not shown). In addition, a previously unknown fragment, *Bam*HI f, was observed in the clone p23. (The p13 clone, shown here, contained an impurity which was subsequently removed by standard procedures before proceeding to the transfection studies.) ***b***, Schematic diagram showing the location of DNA from cosmid clones p65, p5, p39, p23 and p31 relative to the *Eco*RI and *Bam*HI physical maps of the 170-kb linear EBV virion DNA[26,27]. The location of DNA from another clone, p33, is also indicated. (An original 'mini-prep' analysis of clone p33 mistakenly suggested that it contained *Bam*HI R; it was therefore used in transfection experiments (Fig. 2) together with p13 in order to provide overlap information with p23. Its corrected sequence, based on hybridization analysis, is shown.) The dashed vertical lines indicate the 9-kb sequence (*Bam* D→T) which clones p13 and p31 have in common. Contiguous sequences from these two clones comprise about one-third of the viral genome.

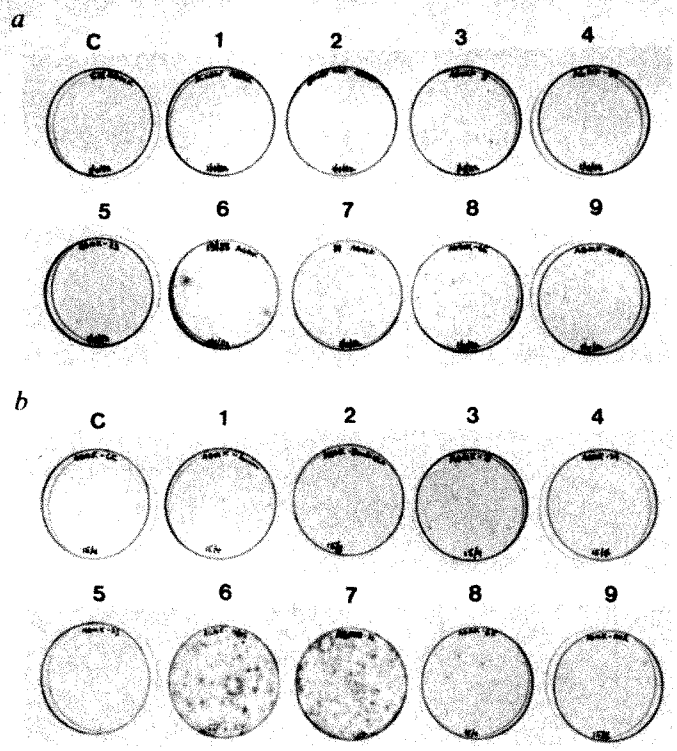


Fig. 2 Dense focus assay of AGMK cells exposed to different fragments of EBV DNA. Subconfluent dishes (50 mm) of early passage AGMK cells were transfected with EBV DNA as indicated below, in the presence of calf thymus carrier DNA (5 μg per dish) and calcium phosphate[28]. Cells were left for 6 h at 37 °C in Dulbecco's modified Eagle's medium (DMEM)–3% glutamine–5% fetal calf serum, then the medium was removed and fresh medium added. After 6 weeks in culture, with weekly changes of medium, cells on each dish were trypsinized and re-plated at a 1:5 dilution onto 50-mm dishes. Thereafter, cells were grown at 37 °C and the media were changed weekly. Individual dishes shown contained AGMK cells transfected with calf thymus (carrier) DNA alone (C) or with linear virion DNA[22] (1); episomal DNA[8] from Daudi cells (2); DNA from clones p5 (3), p39 (4), p23 (5), p13+p33 (6), p31 (7), and p65 (8); and a mixture of cloned DNAs (9). After subculturing, the dishes were left for 4 weeks in culture and 'foci' (*a*) were visualized using Leishman's strain. Whereas aggregations of cells or minor foci were observed at this time in a number of cases, notably among those cells transfected with DNA from the clone p65, p31 or with a mixture of all the cloned DNAs, large distinct foci were observed only in the dish of AGMK cells transfected with the p13/p33 DNA clones. After a further 2 weeks in culture (*b*), large densely growing cell colonies were evident in both the p13/p33 (6) and p31 (7) experiments. EBV DNA was used at a concentration of 5 μg per dish, except in the 'mix' experiment (9), where the total amount of viral DNA was ~25 μg. Failure to form foci in the latter experiment may be a consequence of 'poison sequences' in the cosmid at the concentration used[29].

obtained were not observed when uncleaved material was subjected to similar analyses. In very early passage cultures of 13/33 f4 cells, four bands hybridized to viral DNA (Fig. 4*a*); in later passages of the same cell line, the two largest bands were replaced by a single band having an electrophoretic mobility intermediate between the *Bam*HI co-migrating fragments B/C and D/E (see Fig. 4*b–d*). No further change in the DNA was observed in cells carried for more than a year *in vitro*. These data suggest that some sequence alteration may have occurred between the initial integration event(s) and the establishment of the cells in culture. The two smallest fragments initially observed (Fig. 4*a*) may be derived from monkey DNA as, unlike the larger fragments, their hybridization with EBV DNA could be competitively inhibited by prehybridization with excess cold AGMK DNA. Although cross-hybridization with monkey DNA has not previously been reported, it is well documented that Epstein–Barr virion DNA has regions of homology with a variety of other eukaryotic DNAs[12,13].

Examination of the 13/33 and 31 cell lines for the expression of EBNA proved negative, even though the genetic information for this antigen could at least theoretically have been present in the 13/33 lines (but not in the 31 lines)[14]. Negative results for EBNA were also obtained in the laboratories of G. Klein and D. Crawford (personal communications).

The results of our experiments are consistent with the notion that a specific sub-genomic region of the Epstein–Barr viral

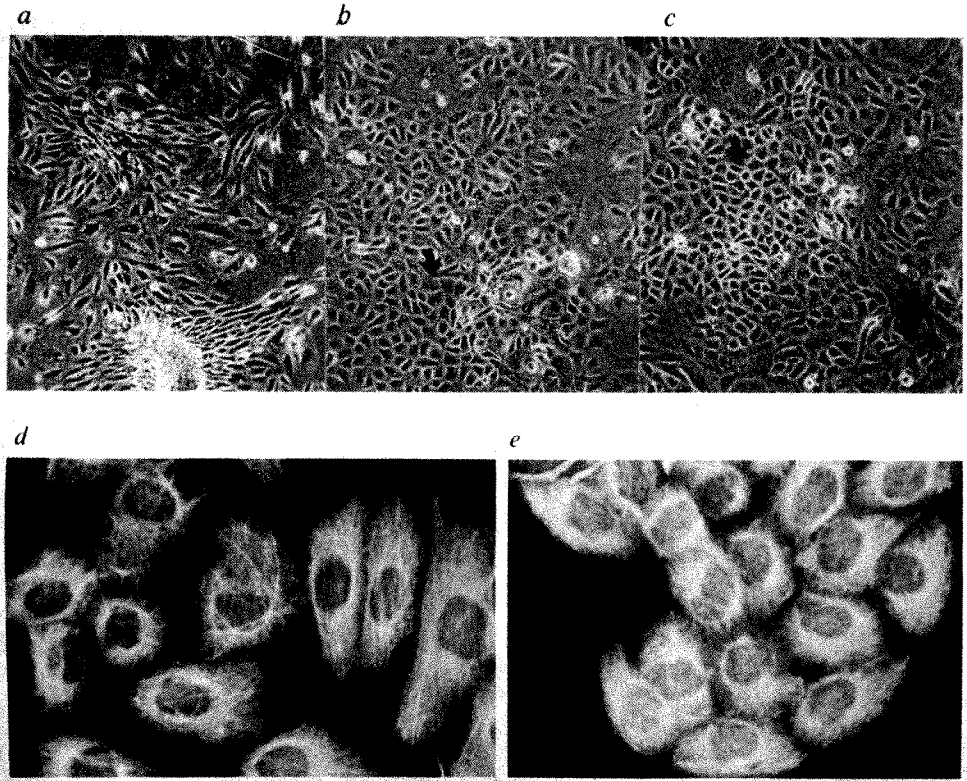


Fig. 3 Morphology of immortalized cells. *a–c*, Light micrographs showing heterogeneous population of cells observed in an early culture of AGMK cells (*a*) and in dishes containing the cuboidal epithelial cells present in the line 13/33 f4 after 14 (*b*) or 34 (*c*) weeks in culture. 13/33 f4 cells examined at an earlier time (4 weeks) showed no obvious morphological differences from the 14- or 34-week cultures. Living cells shown by phase-contrast optics. ×48. *d*, *e*, Immunofluorescent staining with the monoclonal antibody LE61 which recognizes kidney epithelium[9], showing the tonofilament organization of stained cells. Subconfluent cells from the 31 f4 cell line (*d*) are shown, together with the typical close-packing arrangement of rapidly growing cells (*e*), as exemplified by cells from the 13/33 f4 line.

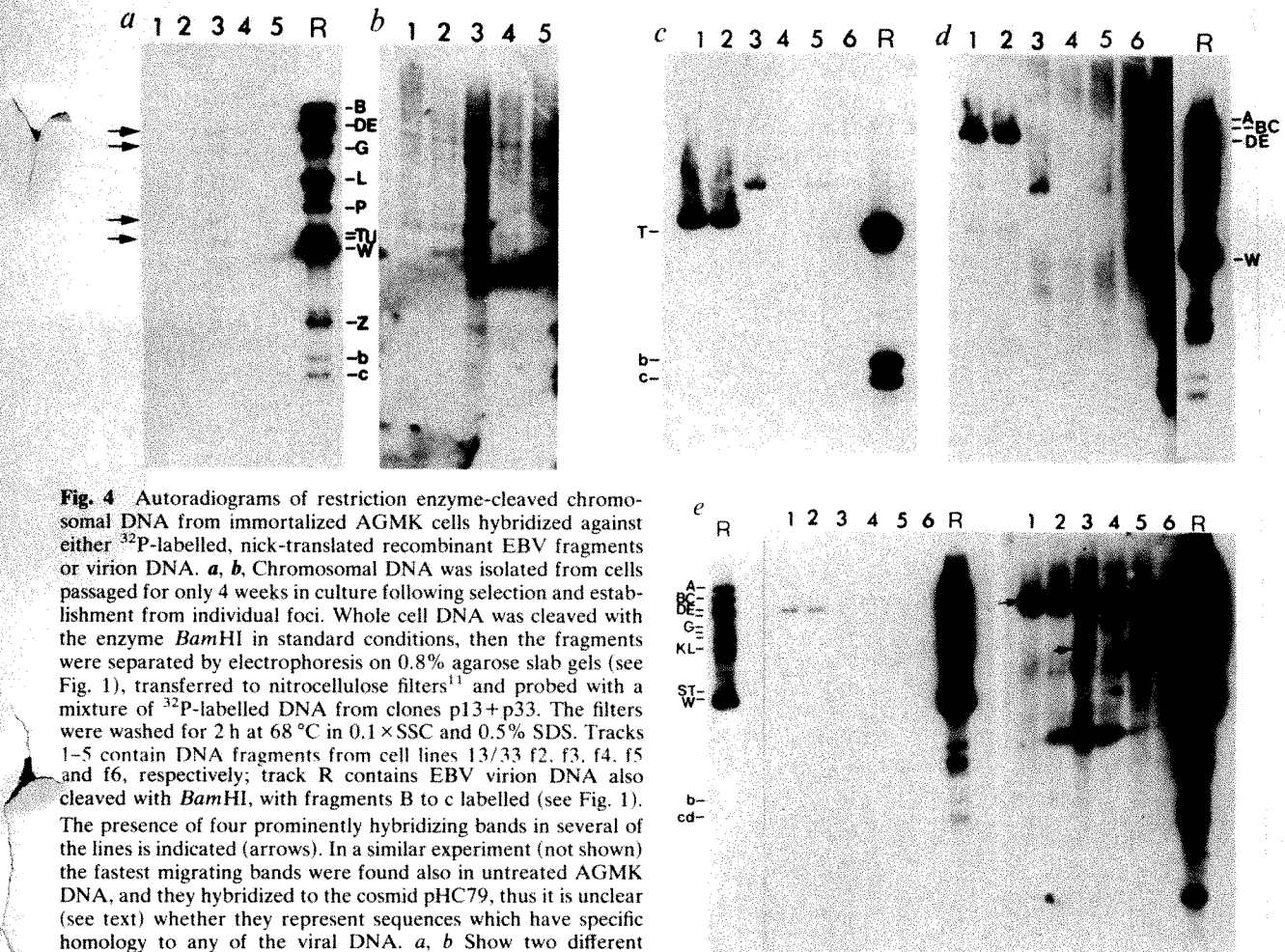


Fig. 4 Autoradiograms of restriction enzyme-cleaved chromosomal DNA from immortalized AGMK cells hybridized against either ^{32}P-labelled, nick-translated recombinant EBV fragments or virion DNA. ***a***, ***b***, Chromosomal DNA was isolated from cells passaged for only 4 weeks in culture following selection and establishment from individual foci. Whole cell DNA was cleaved with the enzyme *Bam*HI in standard conditions, then the fragments were separated by electrophoresis on 0.8% agarose slab gels (see Fig. 1), transferred to nitrocellulose filters[11] and probed with a mixture of ^{32}P-labelled DNA from clones p13+p33. The filters were washed for 2 h at 68 °C in 0.1×SSC and 0.5% SDS. Tracks 1–5 contain DNA fragments from cell lines 13/33 f2, f3, f4, f5 and f6, respectively; track R contains EBV virion DNA also cleaved with *Bam*HI, with fragments B to c labelled (see Fig. 1). The presence of four prominently hybridizing bands in several of the lines is indicated (arrows). In a similar experiment (not shown) the fastest migrating bands were found also in untreated AGMK DNA, and they hybridized to the cosmid pHC79, thus it is unclear (see text) whether they represent sequences which have specific homology to any of the viral DNA. *a*, *b* Show two different time exposures. In the longer exposure (*b*), minor bands can be observed, particularly in track 3 which corresponds to material from the cell line 13/33 f4. ***c***, ***d***, Analysis of DNA from cells passaged continuously for at least 6 months in culture. Tracks 1–5 contain *Bam*HI-cleaved whole cell DNA from lines 13/33 f4, f5 and f6, 31 f4L and 13/33 (uncloned), respectively, and tracks 6 and R contain cleaved DNA from control AGMK cells and EBV virions, respectively. In *c*, the probe was the nick-translated EBV fragment *Eco*RI H, carried on plasmid pAT153 (ref. 24), which hybridized to EBV DNA *Bam*HI fragments T, b and c, as shown (track R). *d*, The results obtained when the blot was washed at 68 °C overnight in low-salt conditions, then re-probed with nick-translated virion DNA and exposed for a longer time. The strongly hybridizing bands in tracks 1 and 2 (in *c*) are essentially no longer visible, whereas the weakly hybridizing bands (see tracks 3 and 5) remain. In addition, in *d*, a new band having a mobility intermediate between *Bam*HI co-migrating fragments B/C and D/E can be seen, especially in tracks 1 and 2. These data suggest that EBV DNA is present but that the strong bands seen in *c* cannot be exclusively correlated with viral DNA. A comparison of *c* and *d* with *a* and *b* further suggests that some change in sequence may have occurred during the passaging of the cells. Most notable is the apparent loss of the two most slowly migrating bands, with their possible replacement by a single, slowly migrating band in lines 13/33 f4 and f5 (tracks 1 and 2), and a faster migrating band in line 13/33 f6 and uncloned 13/33 cells (tracks 3 and 5). ***e***, An experiment analogous to that described for *c* and *d*, but with ^{32}P-labelled nick-translated virion EBV DNA used as the initial probe. Two exposures are shown, and the major hybridizing bands are indicated by arrows. Whole cell DNA isolated from 13/33 f4 cells after more than a year in culture gave a result (not shown) indistinguishable from that found in track 1, indicating sequence stability and the continued presence of the viral DNA during cell propagation.

genome overcomes the senescence observed in untreated AGMK cells and confers the property of unlimited growth on the monkey epithelial cells in culture. Cells immortalized with the overlapping p13/p33 and p31 fragments, following propagation from isolated dense foci, had a finite capacity to grow in soft agar, however, and failed to produce tumours in whole animal (nude mice) experiments. Thus, it seems that of the multi-stage events necessary for the production of malignant cells[15], not all have occurred in these epithelial cells. Note that normal human B lymphocytes, immortalized by EBV infection, initially also fail to produce tumours in nude mice[16]. The most obvious explanation for these data is that EBV *per se* lacks gnant capacity, and additional 'helper' functions, such as e derived from chemical carcinogens or human oncogenes, necessary for inducing a final, transformed state of the cell. Our experiments have produced cell lines which should allow this conclusion to be explored *in vitro*.

The original choice of AGMK cells as an experimental system was based on the fact that expression of Epstein–Barr viral functions has been observed in many non-human primates[17–19] and that transformation efficiency, a possible reflection of stable integration of heterologous information, might be expected to be high[20]. The main cautionary note to be considered in interpreting our data resides in the fact that an endogenous 'EBV-like' virus has been isolated from a lymphoblastoid African green monkey line (AGM-1106) by zur Hausen and colleagues[21]. While this lymphotropic virus would not be expected to be present in kidney cells, as EBV DNA has never been found in human kidney epithelial cells, it is conceivable that AGMK cells might already carry herpesvirus information that predisposes them to immortalization. The EBV sequences from the overlapping p13/p33 and p31 clones were unique, however, among the recombinant and other DNAs introduced during the experimentation protocol in producing immortalization (see Fig. 2). Competition experiments have shown that these sequences are absent from the original AGMK cells and that they survive in the established epithelial lines. Thus, they are undoubtedly important in the immortalization event.

Similar experiments are being done to study the interaction of fragments of EBV with other primate cells. Preliminary data

using primary kidney cells from common marmosets (*Callithrix jacchus*) and primary human breast cells (studies with N. Wedderburn and S. E. Chang) show that at least one of the EBV cloned DNA fragments (p 31) which confers immortality on AGMK cells, also markedly promotes growth in these cells. As these studies are potentially relevant to the human disease, nasopharyngeal carcinoma, the experimental approach should be extended to a variety of human epithelial cells.

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Initiation of translation at internal AUG codons in mammalian cells

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Initiation of translation of eukaryotic mRNAs typically occurs at the first AUG triplet from the 5′ end of the message, although several notable exceptions have been described[1–3]. Using vectors which efficiently express the gene encoding the surface antigen of hepatitis B virus in monkey cells, we have studied the consequences of inserting ATG triplets in all three reading frames upstream of the usual translational initiation codon of this gene. In agreement with the scanning model for eukaryotic translation initiation[1,2], these additional codons can severely depress the initiation of translation at the 'authentic' start codon, although the extent of inhibition depends on sequences flanking the upstream AUG. Such inhibition can, however, be at least partially suppressed by the presence of a translation termination codon in-frame with the upstream AUG. These results raise the possibility that mammalian ribosomes can reinitiate translation at an AUG codon after previously initiating, and terminating, at an upstream site.

We have used a rapid expression assay to quantify levels of the surface antigen of hepatitis B virus (HBsAg) production

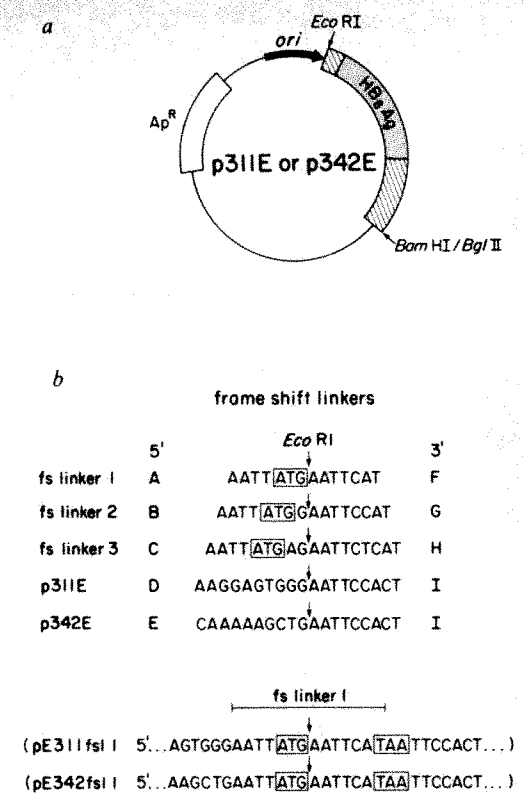


Fig. 1 Construction of pE311fsl and pE342fsl plasmids. The pE311fsl plasmids were derived from p311E (ref. 4), containing the 2,000-bp *Eco*RI–*Bg*III fragment of hepatitis β virus (ref. 9) inserted into plasmid pML (ref. 18) between the *Eco*RI and *Bam*HI sites and following the 311-bp *Eco*RII fragment of SV40 oriented in the early direction. This SV40 fragment spans the replication origin (nucleotides 5,092–160 (ref. 17)) and the early viral promoter[5]. The pE342fsl plasmids were derived from p342E (ref. 4), identical to p311E except that a 342-bp *Pvu*II–*Hin*dIII fragment (nucleotides 5,171–270 (ref. 17)) spanning the SV40 origin was used instead of the 311-bp *Eco*RII fragment. Both plasmids were modified by incompletely digesting with *Eco*RI and filling-in the restriction site using DNA polymerase as described previously (ref. 19). Plasmids were screened for the loss of the *Eco*RI site preceding the SV40 early promoter. Into the single remaining *Eco*RI site immediately following the SV40 promoter were separately inserted the three oligonucleotides, synthesized by the phosphotriester method[20]. These oligonucleotides are bounded by the self-complementary ends of the *Eco*RI site and contain an ATG triplet followed by an *Eco*RI site (*b*). The region spanning the *Eco*RI site used in the construction of every plasmid was directly sequenced using the dideoxynucleotide chain termination method[21] to verify the structure of the linker and the reading frame. *a*, Schematic map of plasmids p311E and p342E. The HBsAg gene is denoted as a shaded box. Nontranslated regions are cross-hatched and the coding region is shown as a stippled box. The position of the SV40 origin/promoter fragment[4,5] is shown. *b*, Sequence of the three oligonucleotide frameshift linkers. The sequences of the oligonucleotide linkers are shown as a 5′ component (A, B, C) and a 3′ component (F, G, H). D, E and I illustrate the sequence at the *Eco*RI site present at the junction of the SV40 and HBV inserts in p311E and p342E (ref. 4). The resulting sequence spanning the *Eco*RI site of pE311fsl-1 and pE342fsl-1 is shown.

directed by mutants of plasmids p342E and pE311 (ref. 4). These pBR322-based plasmids contain the gene encoding HBsAg, positioned behind either of two different, but overlapping, Simian virus 40 (SV40) DNA fragments which contain sequences spanning the viral origin of DNA replication and the early promoter (ref. 5) (Fig. 1*a*). When introduced into COS cells (monkey cells which synthesize SV40 T antigen[6]), plasmid replication ensues with the concomitant synthesis of significant levels of HBsAg mRNA[4,7]. We have previously demonstrated by S_1 nuclease analysis that such mRNA transcribed from both

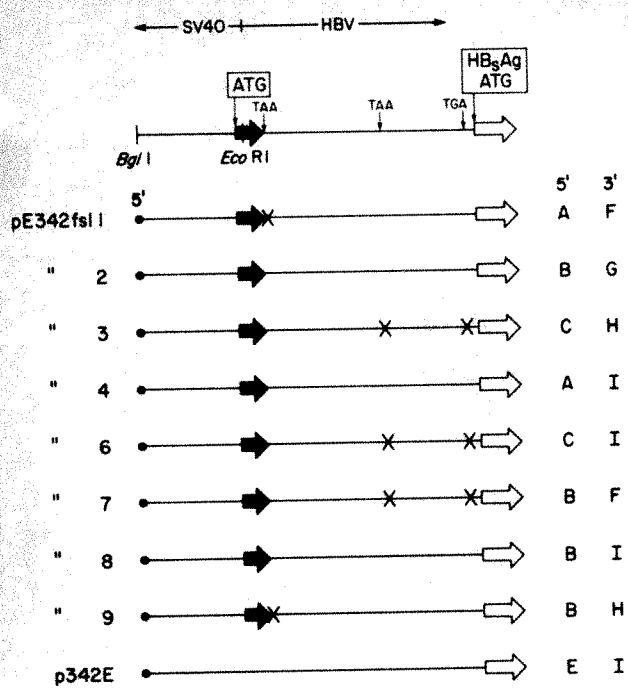


Fig. 2 Structure of the p342E frameshift plasmids. Plasmids p342E, pE342fsl-1, -2 and -3 were digested with *Eco*RI and *Sal*I, the two resulting fragments separated and purified on an acrylamide gel using standard procedures[19]. The additional hybrid plasmids were constructed by ligation of the appropriate fragments. The structure of each was verified by direct determination of the nucleotide sequence across the restored *Eco*RI site[21]. The sequence of the SV40/hepatitis B virus junction is listed as a 5′ and 3′ component. The corresponding nucleotide sequence can be deduced from the information provided in Fig. 1*b*. X, The locations of termination codons in-frame with the linker ATG. The position of the linker ATG and the translation start codon of the HBsAg gene are denoted above the line drawing.

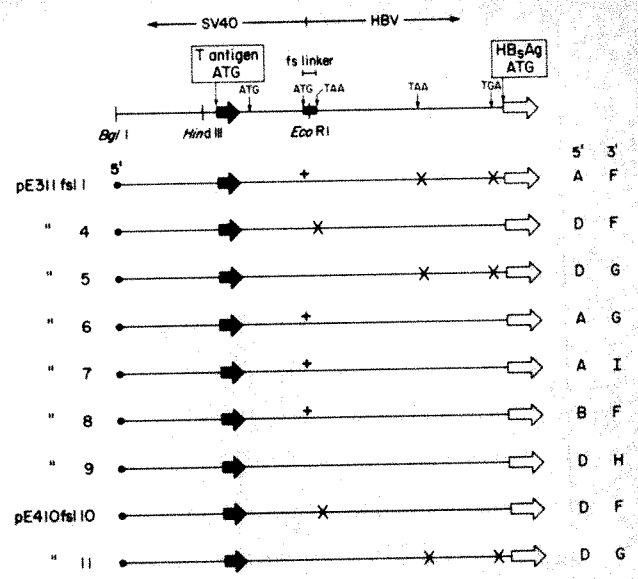


Fig. 3 Structure of the p311E frameshift plasmids. Plasmids p311E and pE311fsl-1, -2, and -3 were treated as described in Fig. 2 and the various combinations constructed as indicated. All junctions at the restored *Eco*RI site were verified by direct sequence analysis. Plasmids pE410fsl-10 and -11 were constructed by digesting p311E with *Bgl*I and *Sal*I, and inserting the 2,400-bp fragment spanning the HBsAg gene between the equivalent sites of p342E (see also Table 2). Symbols are as described in Fig. 2; the presence of an ATG provided by the frameshift linker is indicated by a '+'. The location of the possible termination codons are also shown. The precise structure of the junction can be deduced from the sequences shown in Fig. 1*b*.

plasmids initiates within the SV40 promoter at the expected distance from the TATA box[4] and terminates within HBV sequences 1,100 nucleotides 3′ of the coding region of the HBsAg gene (ref. 7, and C.-C.L., C.C.S. and A.D.L., unpublished data). In pE342, the gene encoding the surface antigen is positioned directly 3′ of SV40 sequences encompassing the complete early promoter[4], such that no AUG triplets precede the translation initiation codon of HBsAg in the mRNA encoding HBsAg (ref. 4 and Fig. 1*a*). COS cells transfected with this plasmid synthesize and secrete significant quantities of HBsAg (ref. 4), a protein of 226 amino acids[8,9] whose synthesis and secretion proceeds without the involvement of a cleavable signal peptide[4,10,11].

To assess directly the effects of upstream AUG triplets on downstream translational initiation, we inserted one of three synthetic *Eco*RI DNA linkers (fsl-1, -2 and -3, Fig. 1*b*), each containing an ATG triplet, into the unique *Eco*RI site of p342E (located at the junction of the SV40 and HBV sequences), to generate pE342fsl-1, pE342fsl-2 and pE342fsl-3. Note that insertion of each of these linkers introduces an upstream ATG triplet in a particular reading frame relative to the 'authentic' translational initiation codon of HBsAg (Fig. 2). These linkers contain the recognition sequence for the restriction endonuclease *Eco*RI; thus after insertion into the vector, a unique *Eco*RI site is restored. This facilitated the construction of hybrid vectors (pE342fsl-4, -6, -7, -8 and -9, Fig. 2), such that the effects of the reading frame of the upstream AUG triplet relative to the 'authentic' HBsAg initiation codon on HBsAg expression could be assessed independently of small sequence differences within the linker (for example, the nucleotide at position +4 relative to the 5′ AUG (ref. 2)) which distinguish the three frameshift linkers (Fig. 1*b*). The effect of these insertions on HBsAg expression was evaluated by transfecting COS cells with the various plasmids and assaying HBsAg in the medium 72 h later. Table 1 illustrates that many of the frameshift plasmids direct the synthesis of HBsAg at levels comparable to p342E, confirming that upstream AUG triplets do not necessarily impair internal initiation[12]. These results extend previous descriptions of this phenomenon[12] since they include AUGs flanked by 'favourable' nucleotides[2,3] at the −3 (A) and +4 position (G) (compare pE342fsl-7 and -9 with p342E). In contrast, plasmids pE342fsl-2, -4 and -8 direct the synthesis and secretion of somewhat lower amounts of HBsAg. As illustrated in Table 1, a common feature of these plasmids with a reduced ability to express HBsAg is the absence of an in-frame translational termination codon intervening between the AUG triplet within the synthetic linker and the AUG triplet defining the start of the HBsAg gene, suggesting that the presence of such a termination codon may enhance internal initiation at the HBsAg gene.

These results demonstrate that, although the presence of an upstream AUG triplet is not in itself sufficient to preclude translation initiation from an internal site (see also refs 13, 14), certain configurations of such a triplet can suppress internal initiation. To see whether flanking sequences could modulate the efficiency with which ribosomes recognize AUG codons as initiation sites[2,3], we examined the effects of inserting an 'authentic' translation initiation codon 5′ of the gene encoding HBsAg, using plasmid p311E (ref. 4), which, although similar to pE342, contains additional sequences 3′ of the SV40 early promoter, including the translational initiation site for SV40 T antigen. As we have reported, plasmid p311E is severely restricted in its ability to promote the synthesis and secretion of HBsAg synthesis[4]. Previous evidence indicated that this block is not a consequence of transcriptional impairment resulting from the absence of the enhancer element associated with the 72-base pair (bp) repeat unit within the SV40 promoter[4], suggesting that HBsAg is translated very poorly, if at all, from its message. This was confirmed by examining HBsAg synthesis from additional plasmid constructions (p232E and p410E). Plasmid

Table 1 Expression of pE342fsl plasmids in COS cells

Plasmid	Linker* sequence 5′	3′	Reading† frame	Termination‡ codon	HBsAg§
pE342fsl-1	A	F	+	+	17,145 ± 1,925
pE342fsl-2	B	G	same	−	9,175 ± 20**
pE342fsl-3	C	H	−1	+	23,620 ± 1,640
pE342fsl-4	A	I	same	−	6,235 ± 1,235
pE342fsl-6	C	I	−1	+	17,440 ± 1,260
pE342fsl-7	B	F	−1	+	19,645 ± 550**
pE342fsl-8	B	I	+1	−	1,980 ± 280
pE342fsl-9	B	H	+1	+	22,580 ± 580
p342E	E	I	−	−	25,785 ± 5,560
p232E‖	E	I	−	−	16,000 ± 600**

Confluent 60-mm dishes of COS-1 cells[6] were transfected in duplicate with 0.5 μg plasmid DNA using a modified DEAE-dextran method[16] as described elsewhere[4]. After 16 h, the cells were washed once with Dulbecco's modified Eagle's medium (DMEM), then fed with 3 ml 7% calf serum in DMEM. Two days later, the cells were re-fed with fresh media. After 24 h, the amount of HBsAg secreted into the medium was quantitated by RIA using the Ausria II kit (Abbott).

* Sequence of frameshift linker as described in Fig. 1 legend.

† Reading frame of linker ATG relative to that of the HBsAg.

‡ Presence (+) or absence (−) of a termination codon in frame with the upstream ATG.

§ Counts per minute as assayed by Ausria II kit, corrected for included negative control. Results are averaged values (with ranges given) obtained from four (** or two) independent experiments.

‖ p232E was constructed by exchanging the proximal promoter sequences of p311 (which lack the SV40 72-bp repeats) with those of pE342 (which lack SV40 T antigen coding information) at the *Bgl*I site. p232E thus contains neither an intact 72-bp repeat nor the translational initiation codon of SV40 T antigen (SV40 sequences extending from the *Eco*RI site at position 160 to the *Hin*dIII site at position 5,171 (ref. 17)).

Table 2 HBsAg expression of pE311fsl plasmids in COS cells

Plasmid	Linker* sequence 5′	3′	Reading† frame	Termination‡ codon	HBsAg*
p311E	D	I	same	−	60 ± 50
pE311fsl-1	A	F	−1	+	1,290 ± 205
pE311fsl-4	D	F	+1	+	2,725 ± 310
pE311fsl-5	D	G	−1	+	1,350 ± 155
pE311fsl-6	A	G	same	−	90 ± 45
pE311fsl-7	A	I	+1	−	460 ± 35
pE311fsl-8	B	F	same	−	20 ± 25
pE311fsl-9	D	H	same	−	125 ± 10**
p410E§	D	I	same	−	30 ± 30
pE410fsl-10	D	F	+1	+	2,895 ± 105**
pE410fsl-11	D	G	−1	+	2,130 ± 180**

Methods of transfection and assay are as described in the legend to Table 1.

* Headings are as defined in Table 1.

† Reading frame of T-antigen translation initiation codon relative to that of HBsAg initiation codon.

‡ p410E was created by the exchange of the proximal SV40 promoter sequences of p342E with the distal promoter sequences of p311E using the SV40 *Bgl*I site. p410E thus contains both SV40 72-bp repeats as well as the translational initiation codon of SV40 T antigen (SV40 sequences from the *Pvu*II site at position 270 to the *Eco*RII site at position 5,092 (ref. 17)).

p232E lacks sequences comprising the 72-bp repeat, but is otherwise identical to pE342. p232E and p342E produce similar levels of HBsAg (Table 1) consistent with the interpretation that the HBV genome contains enhancer sequences capable of complementing those of SV40 (ref. 4), while the insertion of the SV40 enhancer sequences into p311E (generating p410E) fails to restore HBsAg synthesis (Table 2). Thus the inability of p311E to promote efficient HBsAg synthesis appears to result from an extreme inhibitory effect of the SV40 T-antigen translation initiation codon located 231 nucleotides 5′ of the AUG codon which normally initiates HBsAg synthesis.

Protein synthesis initiating at the first AUG (that of T antigen) from mRNA directed by p311E would be expected to produce a hybrid polypeptide consisting of the first 24 amino acids of SV40 T antigen, 52 amino acids specified by the sequence immediately preceding the HBsAg gene, and 226 amino acids of HBsAg (ref. 4). Consistent with this interpretation, intracellular extracts of COS cells transfected with p311E do contain an extended form of HBsAg as determined by radioimmunoassay (RIA) and immunoprecipitation (data not shown), indicating that a polypeptide with HBsAg determinants is synthesized in a form which cannot be secreted. To examine this phenomenon in more detail, the frameshift linkers were incorporated into the unique *Eco*RI site of the parent plasmid p311E or p410E (Fig. 1), and pertinent hybrid plasmids were prepared (Fig. 3). The ability of the various constructs to promote HBsAg synthesis was determined by assaying the medium of transfected COS cells 72 h after DNA administration.

Table 2 illustrates that, as with pE311, no secreted HBsAg is observed with any frameshift linker plasmid expected to generate a fused T antigen/HBsAg polypeptide. In marked contrast, however, significant levels of HBsAg are synthesized from those plasmids which contain an in-frame translational termination codon following the translational initiation codon of SV40 T antigen. This effect was independent of the presence of an AUG triplet within the linker sequence (compare pE311fsl-1 with pE311fsl-5). Specifically, consider that plasmids pE311fsl-6, -8 and -9 all produce less than 1% control levels (that produced by p232E) of HBsAg. These three plasmids are not identical, yet in each the 5′ AUG is present in the same reading frame as the surface antigen AUG, with no intervening in-frame termination codons. Plasmid pE311fsl-7 produces less than 3% of the control level of HBsAg; like the others it lacks an in-phase termination codon, but differs by having the SV40 T antigen initiator AUG in the second reading frame relative to the surface antigen AUG. In contrast, those plasmids (both p311E and p410E-based) in which an in-frame termination codon intervenes between the 5′ AUG and the AUG encoding the first amino acid of HBsAg direct the synthesis of significantly more HBsAg. Interestingly, the presence of an in-frame translational termination codon following the upstream AUG does not restore levels of HBsAg produced by the pE311 vectors to those produced by p342E or p232E (compare Tables 1 and 2). This could be due to the inability of a termination codon to promote the highly efficient use of a downstream initiation codon; alternatively the structure of the hybrid T antigen/HBsAg mRNA may preclude efficient translation of the HBsAg gene.

It has previously been proposed that nucleotides in positions −3 (A) and +4 (G) facilitate recognition of the AUG codon by eukaryotic ribosomes[2]. Our results suggest that additional sequences may be involved in this process, since internal initiation occurs much less frequently when an 'authentic' AUG is positioned upstream of the HBsAg gene (pE311 and pE410-based vectors) than when synthetic AUGs conforming to these specifications are present upstream (pE342-based vectors). The nature of such sequences is uncertain, but the possibility remains that a 'functional' equivalent of a ribosome binding site exists, despite evidence that most mRNAs do not reveal any indication of a specific base-pairing ability between their 5′ non-coding segments and the 3′ terminus of 18S rRNA (ref. 15).

The fact that internal initiation at an otherwise suitable AUG triplet can be thoroughly suppressed by an upstream AUG codon lends strong support to the scanning model for eukaryotic translation initiation[1,2], and is inconsistent with the concept that ribosomes bind directly to AUG initiator codons in mRNA irrespective of their proximity to the 5′ terminus[12]. In contrast to previous suggestions that internal initiation can occur only when upstream AUG triplets are flanked by unfavourable nucleotides[2], our results suggest that reinitiation of translation can occur after the efficient utilization of upstream AUG codons, provided translation has been terminated before the ribosome has reached the internal AUG. It is unclear what factors dictate the efficiency with which such reinitiation occurs; this may be governed to some extent by the position of the termination codon relative to the flanking AUG codons. The fact that internal initiation does not occur in the absence of such a termination codon in the pE311-based vectors strongly argues that initiation at the internal AUG triplet (in those vectors

containing an intervening termination codon) is not mediated by ribosomes which 'escaped' initiation at the upstream site[2], but rather by true reinitiation, although we stress that in a general sense the two models are not incompatible with one another. For example, internal initiation at a site preceded by an efficiently utilized AUG codon may proceed only when translation from the upstream site has first terminated. However, in those instances where only inefficiently recognized upstream AUG triplets are present, an in-frame termination codon would not be required for efficient translation at the internal AUG, since migrating 40S ribosome subunits may simply bypass a weak AUG. In view of the observation that in-frame termination codons almost always follow upstream AUGs in those messages where translation initiates internally, it has been proposed that such terminators may function to facilitate the early return to circulation of those ribosomes which initiate at the upstream site[2]. The prospect that ribosomes may not be released immediately after translation termination, but rather continue scanning for at least some distance, provides an additional rationalization for this observation, and suggests a mechanism by which polycistronic transcripts may function in mammalian cells.

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Nucleotide sequence of avian retroviral oncogene v-*mil*: homologue of murine retroviral oncogene v-*raf*

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Eukaryotic cells contain genes termed proto-oncogenes (c-*onc*) which have the potential to transform cells in culture and induce tumours *in vivo*. Most of these genes have been identified by their occasional incorporation into retroviral genomes which can act as natural transducing vectors for these and perhaps other cellular genes[1,2]. Cell-derived oncogenes of retroviruses (v-*onc*) are associated mostly with the induction of mesenchymal tumours whereas carcinoma induction is rare[3]. One of these rare carcinoma-inducing viruses is the acutely transforming avian retrovirus MH2 (refs 3–5). Recently we[6,7] and others[8] have shown that this virus carries a novel putative oncogene, v-*mil*, in addition to the known oncogene v-*myc*. While the transforming ability of v-*mil* has not been directly established, we have recently discovered by hybridization analysis that v-*mil* is homologous to v-*raf* (ref. 9), the transforming gene of the murine retrovirus 3611 MSV (ref. 10). Both viruses express the *mil*/*raf* oncogene product as a *gag*-fusion polyprotein[6,11], while the *myc* oncogene of MH2 is expressed via a subgenomic mRNA[12]. Here we report the complete nucleotide sequence of v-*mil* and compare it with that of v-*raf*[34]. The 80% homology between the nucleotide sequences and the 94% homology between the predicted amino acid sequences of the two viral genes clearly indicate that these are the avian and murine forms of the same gene. Comparison of the two sequences with that of the human cellular homologue[13] (T. I. Bonner *et al.*, manuscript in preparation) indicates that v-*raf* has more 3′ untranslated sequences while v-*mil* has additional sequences from two 5′ exons of the cellular homologue. Although the *mil*/*raf* amino acid sequences reveal partial homology to that of the v-*src* product, no tyrosine-specific protein kinase activity is detected for the *gag*–*mil* and the *gag*–*raf* hybrid proteins.

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1,416 nucleotides of MH2 proviral sequence containing the complete v-*mil* sequence as well as adjacent viral *gag* p12 sequence at its 5′ end and *myc* gene sequence at its 3′ end were determined and compared with the corresponding v-*raf* sequence in Fig. 1. The 5′ end of v-*mil* is tentatively located at nucleotide 93 or 94. This determination is based on the observation that nucleotides 1–92 are homologous to the *gag* p12 sequence of Rous sarcoma virus (RSV)[14] while the following nucleotides are not. In addition, comparison with the human homologue c-*raf*-1 shows homology beginning at nucleotide 94. Nucleotides 94–265 are specific to v-*mil*. Comparison with the human cellular homologue indicates that they are derived from three exons, one of which is exon 1 of the chicken cellular clone c-*mil*-4 (refs 7, 15) and two of which were not identified in the chicken clone. Homology to the v-*raf* sequence begins at position 266 and with the exception of two small insertions or deletions continues to the termination codon at nucleotide 1,233. The v-*mil* sequence ends 12 nucleotides 3′ of the termination codon. Beyond this point, the sequence matches that of the *myc* cellular homologue, beginning in the first intron 172 nucleotides 5′ of the second exon (M. Linial, personal communication). The v-*mil* sequence contains an insertion of a nucleotide at 1,209 coupled with the deletion of four nucleotides between 1,215 and 1,218 which preserves the reading frame to the termination codon. The human homologue shows no deletions in this region relative to v-*raf*. Thus, the insertions occurred in the mammalian lineage before the divergence of humans and mice or deletions arose in the chicken lineage. Alternatively, the deletions may have arisen during viral replication of MH2 after its acquisition of the v-*mil* sequence. The nucleotide sequence homology over this common coding region is 80% with most of the changes occurring as silent third position changes so that the predicted amino acid sequences have 94% homology.

The MH2 sequence has only one open reading frame capable of producing the *gag*–*mil* polyprotein of molecular weight 100,000. This reading frame corresponds to the reading frame used to produce the *gag*–*raf* polyprotein. Assuming that the *gag* sequence of MH2 is the same as that of RSV (ref. 14), the predicted polyprotein would contain 514 *gag*-specific amino acids and 380 *mil*-specific amino acids with a calculated molecular weight of 97,014. This agrees well with the molecular weight of the *gag*–*mil* p100 protein determined by SDS–polyacrylamide gel electrophoresis, considering the fact that the protein is phosphorylated and possibly glycosylated. There are three potential glycosylation sites of the form Asn-X-(Thr or Ser) in the *mil* sequence at amino acid positions 16–18, 32–34 and 350–352. Of the 19 amino acid differences between v-*mil*

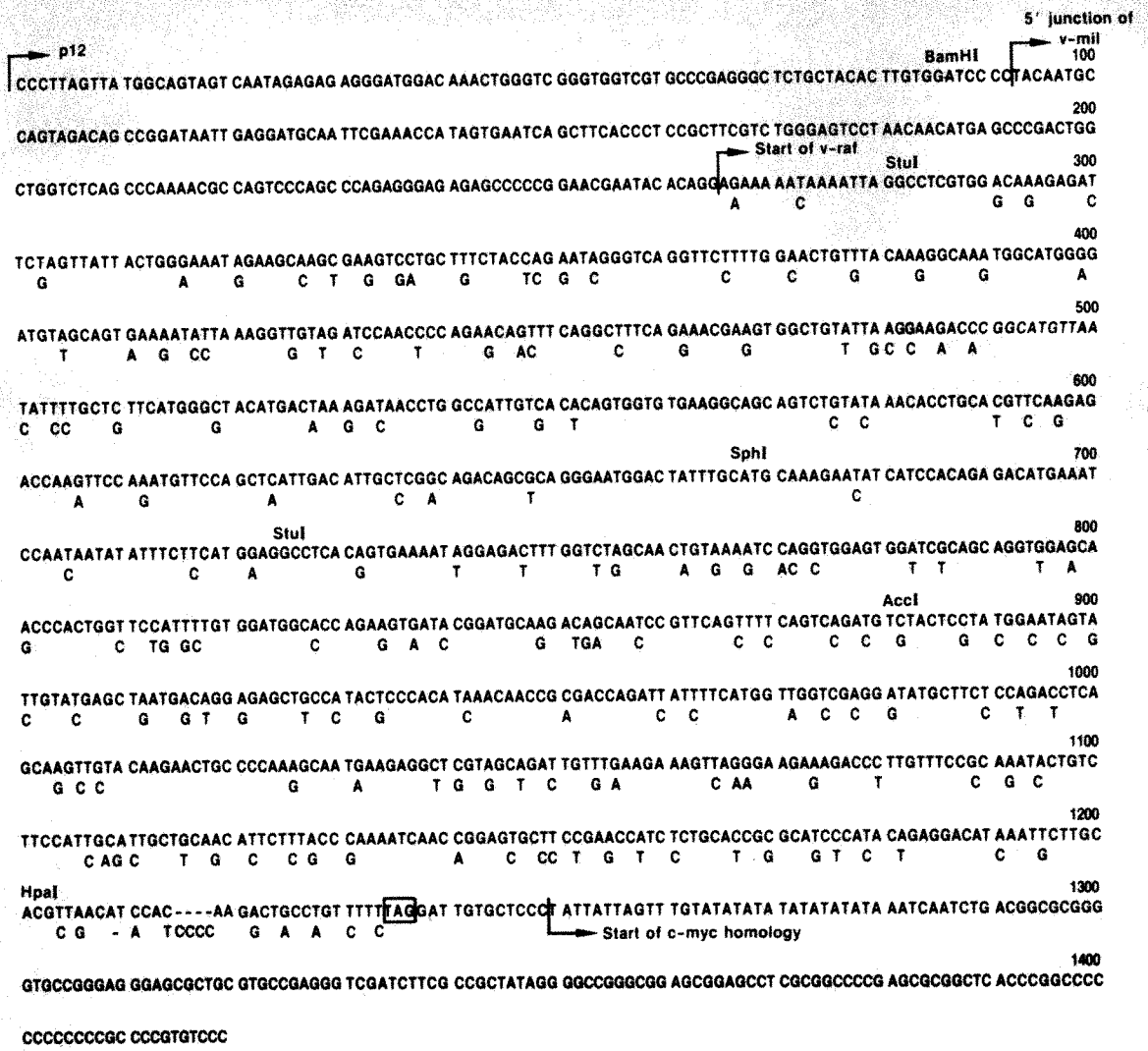


Fig. 1 Nucleotide sequence comparison between the *mil* region of MH2 proviral DNA and v-*raf*[34]. The complete v-*mil* sequence is shown with partial sequences of adjacent viral *gag* and v-*myc* genes. The v-*raf* coding sequence is shown below the v-*mil* sequence at nucleotides where it differs from v-*mil*, and deleted nucleotides are indicated by dashes. The 5′ borders of v-*mil* and v-*raf* are indicated by arrows. The termination codon common to both sequences is boxed. The location of the *mil–myc* junction is indicated by an arrow. Restriction sites used for sequencing of MH2 DNA by the Maxam–Gilbert method[35] are indicated above the nucleotide sequence.

and v-*raf* (Fig. 2), one occurs at a position where v-*mil*, v-*raf* and human c-*raf*-1 differ (T.I.B. *et al.*, manuscript in preparation), 11 occur at a position where v-*raf* and c-*raf*-1 are the same and six occur at a position where v-*mil* and c-*raf*-1 are the same. This result is consistent with mice being more closely related to humans than chickens.

A computer search of known protein sequences revealed that the *mil/raf* oncogene is related to members of the *src* family of oncogenes (see below). The homology is to the carboxy half of the p60 v-*src* product which is associated with tyrosine-specific protein kinase activity. Since the v-*raf* product has not shown any tyrosine kinase activity[11], it was unexpected that this homology exists for the *mil/raf* oncogene. The fact that the v-*mil* protein contains 59 more amino acids than v-*raf* made it important to test the v-*mil* product for kinase activity in comparison with the *raf* protein and with a known tyrosine-specific kinase specified by a member of the *src* family of oncogenes. Figure 3 compares protein kinase activity associated with proteins immunoprecipitated from cells transformed by MH2, 3611 MSV or avian Fujinami sarcoma virus (FSV) carrying the *fps* oncogene[1,2]. Quail embryo cells or rat cells transformed by FSV contain the p140 *gag–fps* protein which is phosphorylated and associated with tyrosine-specific protein kinase activity[16–18] leading to efficient *in vitro* phosphorylation of the p140 protein in a kinase assay (Fig. 3, lanes 1, 3). In the same conditions, very low levels of phosphorus are transferred to the p75 *gag–raf* protein of 3611 MSV or the p100 *gag–mil* protein of MH2 (Fig. 3, lanes 5, 7). Furthermore, phosphoamino acid analysis revealed that this phosphorylation occurred at threonine and serine residues but not at tyrosine residues as in the *gag–fps* protein (data not shown). This agrees with the phosphorylation pattern observed *in vivo* for these proteins[11,17,19]. Whether the low level of *in vitro* phosphorylation of the *raf* and *mil* proteins is an intrinsic activity or due to cellular enzymes present in the immunoprecipitates remains to be determined.

There are three other oncogenes, v-*erb* (ref. 20), v-*fms* (ref. 21) and v-*mos* (ref. 22), which are apparently negative for protein kinase activity yet show homology to the kinase portion of v-*src*. Their cellular locations suggest some diversity of function. The product of v-*erb*-B has been shown to be a membrane glycoprotein[23], possibly functioning as a growth factor receptor[24], whereas v-*fms* encodes a glycoprotein associated with the membranes of endoplasmic reticulum[21]. In contrast, fluorescence assays indicate a diffuse cytoplasmic distribution of the v-*mil*[25] and v-*mos*[26] proteins with v-*mil* possibly being associated with RNA[25]. To determine any similarities among these proteins which may distinguish them from the kinase-positive *src*-related genes, we have compared their amino acid sequences in Fig. 4. Four regions show extensive homology, that is amino acids 18–50, 120–138, 157–183 and 202–247.

```
p12
Pro Leu Val MET Ala Val Val Asn Arg Glu Arg Asp Gly Gln Thr Gly Ser Gly Gly Arg Ala Arg Gly Leu Cys Tyr Thr Cys Gly Ser

Start of v-mil                    10                  20                  30
Pro Thr MET Pro Val Asp Ser Arg Ile Ile Glu Asp Ala Ile Arg Asn His Ser Glu Ser Ala Ser Pro Ser Ala Ser Ser Gly Ser Pro

                  40                  50                  60
Asn Asn METSer Pro Thr Gly Trp Ser Gln Pro Lys Thr Pro Val Pro Ala Gln Arg Glu Arg Ala Pro Gly Thr Asn Thr Gly Glu Lys
                                                                                                  Start of v-raf
                  70                  80                  90
Asn Lys Ile Arg Pro Arg Gly Gln Arg Asp Ser Ser Tyr Tyr Trp Glu Ile Glu Ala Ser Glu Val Leu Leu Ser Thr Arg Ile Gly Ser
                                                  Lys MET                 MET

                 100                 110                 120
Gly Ser Phe Gly Thr Val Tyr Lys Gly Lys Trp His Gly Asp Val Ala Val Lys Ile Leu Lys Val Val Asp Pro Thr Pro Glu Gln Phe
                                                                                                                    Leu
                 130                 140                 150
Gln Ala Phe Arg Asn Glu Val Ala Val Leu Arg Lys Thr Arg His Val Asn Ile Leu Leu Phe MET Gly Tyr MET Thr Lys Asp Asn Leu

                 160                 170                 180
Ala Ile Val Thr Gln Trp Cys Glu Gly Ser Ser Leu Tyr Lys His Leu His Val Gln Glu Thr Lys Phe Gln MET Phe Gln Leu Ile Asp

                 190                 200                 210
Ile Ala Arg Gln Thr Ala Gln Gly MET Asp Tyr Leu His Ala Lys Asn Ile Ile His Arg Asp MET Lys Ser Asn Asn Ile Phe Leu His

                 220                 230                 240
Gly Gly Leu Thr Val Lys Ile Gly Asp Phe Gly Leu Ala Thr Val Lys Ser Arg Trp Ser Gly Ser Gln Gln Val Glu Gln Pro Thr Gly
Glu
                 250                 260                 270
Ser Ile Leu Trp MET Ala Pro Glu Val Ile Arg MET Gln Asp Ser Asn Pro Phe Ser Phe Gln Ser Asp Val Tyr Ser Tyr Gly Ile Val
    Val                                      Asp
                 280                 290                 300
Leu Tyr Glu Leu MET Thr Gly Glu Leu Pro Tyr Ser His Ile Asn Asn Arg Asp Gln Ile Ile Phe MET Val Gly Arg Gly Tyr Ala Ser
                     Ala                 Ala
                 310                 320                 330
Pro Asp Leu Ser Lys Leu Tyr Lys Asn Cys Pro Lys Ala MET Lys Arg Leu Val Ala Asp Cys Leu Lys Lys Val Arg Glu Glu Arg Pro
                 Arg                                 Ile                             Val             Lys
                 340                 350                 360
Leu Phe Pro Gln Ile Leu Ser Ser Ile Ala Leu Leu Gln His Ser Leu Pro Lys Ile Asn Arg Ser Ala Ser Glu Pro Ser Leu His Arg
                                     Glu                                                     Pro
                 370
Ala Ser His Thr Glu Asp Ile Asn Ser Cys Thr Leu Thr —— Ser Thr Arg Leu Pro Val Phe
    Ala                         Ala                 Thr     Pro
```

Fig. 2 The predicted amino acid sequence encoded by v-*mil* compared with that of v-*raf*. The amino acids are numbered beginning with the first v-*mil* specific amino acid. The v-*raf* sequence is shown below v-*mil* where it differs from v-*mil*, and deletions are indicated by dashes.

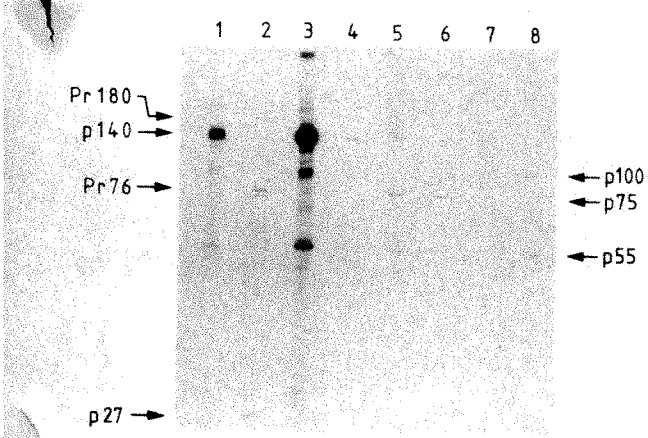


Fig. 3 Protein kinase activity in immunoprecipitates from lysates of cells transformed by FSV, 3611 MSV or MH2. Quail embryo cells transformed by FSV (FAV) (lanes 1, 2), FSV-transformed rat 3Y1 cells[18] (lanes 5, 6), and a nonproducer quail cell clone transformed by MH2 (lanes 7, 8) were used. Lysates were prepared as described previously[16] from unlabelled and ^{35}S-methionine-labelled cells using a lysis buffer containing 50 mM NaCl, 25 mM Tris-HCl *p*H 7.4, 0.5% NP-40, 0.5% sodium deoxycholate, 1 mM EDTA and 400 units ml^{-1} Antagosan (Behringwerke) as a protease inhibitor. Cell lysates were preabsorbed with preimmune rabbit or goat sera and protein A–Sepharose, and immunoprecipitates were then prepared with rabbit antiserum against avian *gag* proteins[6] (lanes 1, 2, 3, 4, 7 and 8) or with goat antiserum against the murine p15 *gag* protein[11] (lanes 5, 6) followed by absorption to protein A–Sepharose. Unlabelled immunoprecipitates were washed with lysis buffer and 20 mM Tris-HCl *p*H 7.4 and were then incubated for 15 min at 30 °C in 50 μl of a kinase reaction solution containing 20 mM Tris-HCl *p*H 7.4 10 mM $MgCl_2$, 1 mM dithiothreitol and 5 μCi [γ-^{32}P]ATP (~2,000 Ci mmol^{-1}; Amersham Buchler, Braunschweig). Reaction products were analysed on a 12.5% polyacrylamide-SDS gel (lanes 1, 3, 5, 7). Immunoprecipitates of ^{35}S-methionine-labelled proteins were analysed in parallel (lanes 2, 4, 6, 8). They include the p140 *gag–fps* protein of FSV (lanes 2, 4), the structural proteins Pr180, Pr76 and p27 of the helper virus FAV (lane 2), the p75 *gag–raf* protein of 3611 MSV (lane 6), and the p100 *gag–mil* protein of MH2 and a possible processing product p55 (lane 8). The autoradiograph was exposed for 12 h.

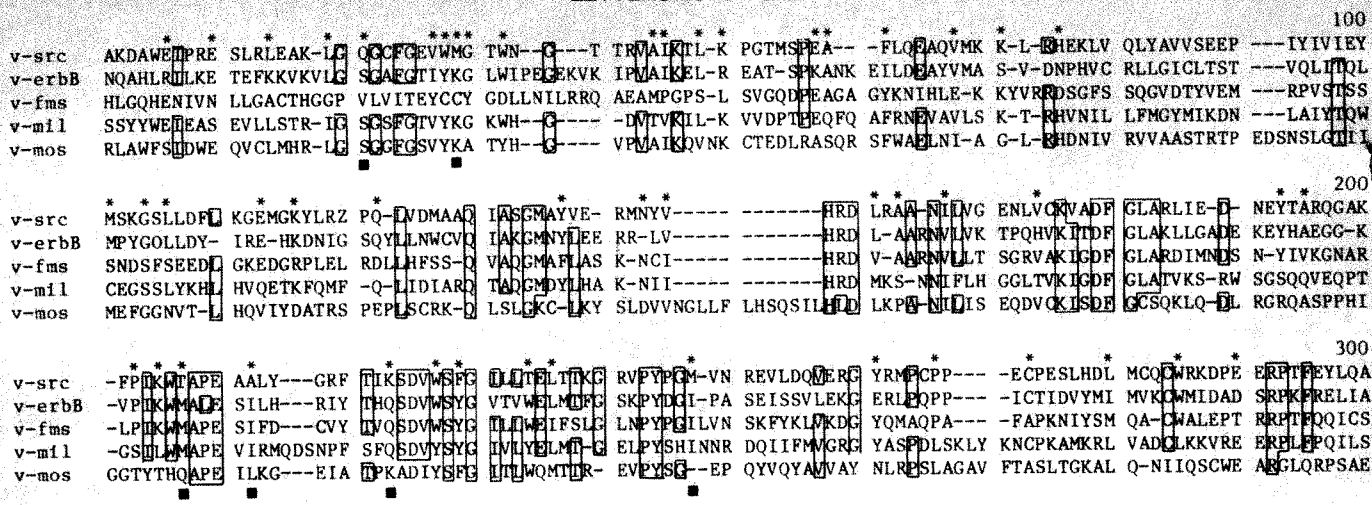


Fig. 4 Alignment of amino acids encoded by non-kinase oncogenes with those encoded by the v-*src* oncogene. The alignment begins at amino acid 71 of v-*mil* and continues to 337. No extensive homology was found outside these limits. Positions at which four or five of the sequences match are boxed. Positions at which three sequences match are indicated by an asterisk above the v-*src* sequence. Positions at which three of the four non-kinase genes match and are different from the kinase positive oncogenes (*fgr, yes, fes, fps, abl* and *src*) are indicated by a closed box below the v-*mos* sequence. The phosphotyrosine of v-*src* occurs at position 193. A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.

Although the other four proteins show substantial homology in the first region, the *fms* sequence shows almost no homology before positions 100–120. The remaining kinase negative proteins (as well as the *raf* protein) contain the sequence Gly-Ser-Gly-X-Phe-Gly-X-X-X-Tyr at positions 21–29 with the serine and tyrosine residues distinguishing them from the kinase-positive oncogenes *src, fgr, yes, fps, fes* and *abl* (refs 14, 27–31). It has been suggested that the Gly-X-Gly-X-X-Gly sequence represents a nucleotide-binding site[32]. A similar sequence, Leu-Gly-Thr-Gly-Phe-Gly-Lys-Val-Val-Glu, is found in v-*fms* about 140 amino acids further towards its amino terminal. The remaining four positions where a common amino acid distinguishes the non-kinase genes, except for *mos*, from the kinase-positive genes occur in the fourth homology region. This region is functionally important as mutagenesis of the alanine at position 211 of v-*src* inactivates its kinase activity[33].

There are three known examples of a retrovirus containing what appear to be two oncogenes: AEV containing *erbA* and *erbB* (ref. 36), E26 containing *ets* and *myb* (refs 37, 38), and MH2 containing *mil* and *myc* (refs 5–7). In none of these cases have both genes been demonstrated to be transforming. The present data demonstrate that v-*mil* has 94% amino acid homology to v-*raf*. When this result is compared with the 96% amino acid homology of v-*raf* to its human homologue c-*raf*-1, it is clear that *mil* is the avian homologue of the murine *raf* gene. As both v-*raf* and the human homologue c-*raf*-1 are capable of transformation, we infer that *mil* is also.

Note added in proof: Close homology has been reported[39] between the *mht* (*mil*) sequence of MH2 and our v-*raf* sequence. The reported *mht* sequence is nearly identical to our *mil* sequence; however, the authors identify the c-*myc* intron sequence as being *mht* 3′ untranslated sequence.

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Seismological evidence for shallow crystalline basement in Southern Uplands, Scotland

HALL *et al.*[1] propose that the Southern Uplands contain crystalline rocks of continental affinity at shallow depths (~1–5 km). We suggest that the same seismic data can, in fact, be used to strengthen the argument for a thick accretionary prism[2].

It has been inferred that prehnite–pumpellyite facies metamorphism in the Southern Uplands is related to tectonic burial and rotation of the various slices of the prism as they were accreted during closure of the Iapetus Ocean, and that isograds in the prism are now subparallel to the present day erosion surface[3–6]. The colour alteration of conodonts in the Northern Belt indicates temperatures between 300 °C and 400 °C (refs 7, 8). The transition of the prehnite–pumpellyite to the greenschist facies lies between 300 °C and 350 °C (ref. 9), thus it has been suggested[4,5] that greenschist facies is present not far below the present day surface over much of the Southern Uplands.

The LISPB seismic profile for the Grampian sector shows P-wave velocities similar to the Southern Uplands Seismic Profile (SUSP[1], that is, 6 km s^{-1} at the surface increasing to 6.05 km s^{-1} at 12 km depth[10]. Mainly amphibolite facies sediments (and granite) occur in the northern part and foliated greenschist facies greywackes occur in the south[11]. In New Zealand, the seismic profile for the Otago greenschist terrane shows a velocity of 6 km s^{-1} for the depth range 1–10 km, lying above a velocity of 6.7 km s^{-1} (ref. 12). In Otago the transition from prehnite–pumpellyite facies to greenschist facies is marked by a transition of poorly foliated greywackes to strongly foliated quartzo-feldspathic schists[13]. Individual mica- and chlorite-schists from Vermont, USA, have experimentally determined velocities (at the appropriate pressures) similar to SUSP[14]. We conclude that the SUSP velocities are compatible with the occurrence of foliated greenschist facies metagreywackes at depths 1–5 km below the present surface.

Velocity anisotropism in foliated metamorphic rocks is well known[12,15] and the 15% anisotropy measured at Eskdalemuir (EKA)[1] is very similar to that in slate, chlorite-schist and mica-schist measured experimentally[14,16] at the appropriate pressure. Similar anisotropism is present at the Broughton array (BTN)[1] if the contribution of arrivals from the Midland Valley is ignored. We conclude that the seismic evidence from the BTN and EKA arrays supports the existence of subvertical, NE–SW foliated greenschists at a shallow depth.

Hall *et al.*[1] reinterpreted the Southern Uplands LISPB sector[10] in terms of block-faulting of crystalline crust. We are not competent to quantify a seismic model involving the block-faulting of a thin layer of isotropic greywackes lying above anisotropic greenschists but we point out that the boundaries between the seismic blocks[1] correspond well with existing block faults[5].

The geological arguments of Hall *et al.*[1] are equivocal: the xenoliths could originate from English continental crust subducted below the Southern Uplands[2]; the Midland Valley conglomerates may have come from the upper 11–17 km of the prism (Cockburnland[17]) which has since been stripped off[4].

We conclude that the seismic evidence supports a thick accretionary prism in the Southern Uplands. We predict that a suitably positioned borehole sited in blocks F2 or F3 will penetrate greenschist facies metagreywackes rather than crystalline continental basement.

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HALL *ET AL.* REPLY—Oliver and McKerrow accept our conclusions that there are crystalline rocks at shallow depth in the Southern Uplands. Their suggestion that the rocks are greenschist facies metagreywackes, part of an accretionary wedge occupying nearly all the present crustal pile, represents an unlikely possibility rather than a probability more central to all the evidence.

Shots on LISPB[1] and the Otago line[2] are too far apart to provide good control on shallow velocities. Inspection of the original LISPB data from shot 1 suggests that the velocity of Dalradian greenschist metasediments is significantly less than 6 km s^{-1} at 1 km depth (in the fast direction—along the horizontal banding of the 'flat belt'). From this and other field measurements across banding, we estimate that at 1 km depth these rocks would have a mean velocity of ~5.6 km s^{-1} and anisotropy of less than 7%. The Otago data may be modelled similarly.

Laboratory data[3–5] show average values for anisotropy in small cores of schists and associated rocks at pressures corresponding to 3 km depth to be only 7–9%, although higher individual values are found. Less anisotropy would be expected in the field. The 15% anisotropy sought by Oliver and McKerrow is likely to require the rocks to have a 40% content of mica[4] oriented consistently throughout the region.

Thus, Oliver and McKerrow's claim that greenschist facies metagreywacke at 1 km depth would have a mean velocity of 6 km s^{-1} and 15% anisotropy is at the limit of possibility.

Turning to the geological evidence, it is unlikely that a 'regional' metamorphic transition from a greywacke with little or no fabric to a wholly reconstituted mica schist occurs over only 1 km depth, despite the 'dynamic' example near the Alpine Fault[6]. Oliver and McKerrow themselves imply elsewhere[7,8] that the transition in the Southern Uplands takes place over a depth range of 7–12 km.

Oliver and McKerrow imply that the accretionary wedge occupies more or less the whole crust north of the subduction zone indicated on WINCH[9] and with at least 10 km already removed by erosion, the stack must have reached a thickness of 40 km or so, far in excess of present-day examples[10].

Xenoliths from post-Caledonide vents in the Southern Uplands are sufficiently similar to those of the Midland Valley to suggest derivation from a similar crust[11]. It has yet to be established if the English continental crust could supply xenoliths of this type, and whether such crust extends far enough north (interpretation of the Iapetus Suture on WINCH suggests that it does not).

Northward translation[12] of the Southern Uplands is required to account for the missing Ordovician fore-arc basin, and to cover the source of the Silurian conglomerates. We cannot seriously entertain the idea that the substantial thickness of Silurian rocks in the southern Midland Valley came from Cockburnland which, in addition, has to supply sediment to a coeval trench to the south yet is rarely more than 5 km wide[12], and occasionally substantially less[13]. The conglomerates

are unlikely to have come from the top of an accretionary pile because the clasts include acid igneous rock fragments (probably first cycle) and foliated greywacke.

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Geomagnetic reversals

RECENTLY Valet *et al.*[1] presented palaeomagnetic data associated with a pair of reverse-to-normal (R–N) polarity transitions from western Crete. The two transition records, differing in age by about 1.2 Myr, were found to possess very similar directional field behaviour in that the corresponding paths of the virtual geomagnetic pole (VGP) were shown to be nearly identical. The authors then noted that an earlier reported N–R VGP path from Crete[2], associated with the transition which closely follows in time the younger of the two R–N reversals, is largely discrepant to the other paths. Given these comparisons, the authors conclude, first, that requirements of the standing field model for transition fields[3] are not satisfied by these data, and second, that the reversal mechanism in the core may be associated with a definite field configuration over some period of time such that successive reversals of the same sense recorded at the same site will be associated with identical VGP paths.

My purpose here is to explore the above argument further. For the authors' assertion to be strictly valid reversals of opposite sense which occur during a time of assumed invariant transitional field characteristics should be observed to possess antipodal VGP paths when recorded at the same site. If, on the other hand, the reversal process is asymmetric such that each transition sense is associated with a different field configuration, then antipodal behaviour probably will not be observed. The question now is: how does the VGP path associated with the N–R transition record[2] compare with the two R–N transition paths[1]?

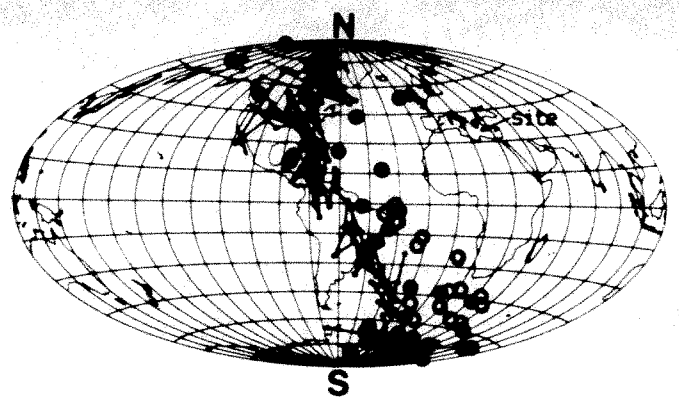


Fig. 1 Paths of the virtual geomagnetic pole associated with three polarity transitions recorded on Crete[1,2]. The drawn path depicts north poles associated with KS06 R–N transition. The solid circles are north poles associated with the older KS02 R–N transition. The open circles are south poles associated with the younger N–R transition (after refs 1, 2).

Figure 1 shows the highly detailed VGP path associated with the younger (KS06) R–N transition[1] along with individual VGPs associated with the older (KS02) R–N transition[1]. Also plotted are the individual south VGPs corresponding to the N–R transition[2]. As can be seen in the figure, all three records contain intermediate VGPs which reside along a relatively narrow path west of the site. Indeed, the longitudinal span of this combined path narrows to less than 45° for near-equatorial poles. Furthermore, an east-to-west migration of intermediate VGPs is a common feature of all three paths, where data exist.

For the R–N north pole transition paths to be found in close proximity to the N–R south pole transition path implies that the north VGP paths of the former transitions are roughly antipodal to the north VGP path of the latter transition. This finding suggests that the harmonic content during all three reversals was quite similar apart from a complete change in sign of the transition field associated with the N–R event. Such a situation is consistent with a reversal process whose time-dependent configurational characteristics are independent of transition sense.

In this regard, the generalized flooding model[4] can successfully accommodate these data through a simulated reversal process which begins and extends from the same localized region in the core for all three polarity transitions from Crete. It is interesting, however, that a pair of recently reported sequential reversals (R–N and N–R) from Kauai, Hawaii[5], have nearly identical north pole transition paths. Such a case cannot be strictly explained by the above arguments, but suggests that major changes in the characteristics of the reversal process in the core can occur abruptly between successive geomagnetic transitions.

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VALET AND LAJ REPLY—We have limited our comparison to two reverse-to-normal (R–N) paths from transitions recorded in the same section. Hoffman remarks that a more complete picture can be obtained using antipodal virtual geomagnetic pole (VGP) paths of N–R transitions. Using the south VGPs relative to a N–R transition recorded in a nearby section at Potamida (which we now call KP02), he concludes that the paths of all three transitions are very similar.

The data for this N–R transition, however, although detailed, are not rigorously classifiable as category 'A' because of lack of points in the second half of the transition[1], as remarked by Hoffman himself[2]. A new detailed sampling with very precise stratigraphic control yields a much more detailed and still unpublished VGP path quite similar to the previous published one, although some differences exist in the equatorial VGPs. A comparison between the KS06 transition VGPs and the south VGPs of this new record is shown in Fig. 2. We believe that this figure shows that the R–N paths from Skouloudhiana and the South Pole N–R path from Potamida are not identical (the difference between the two is smaller

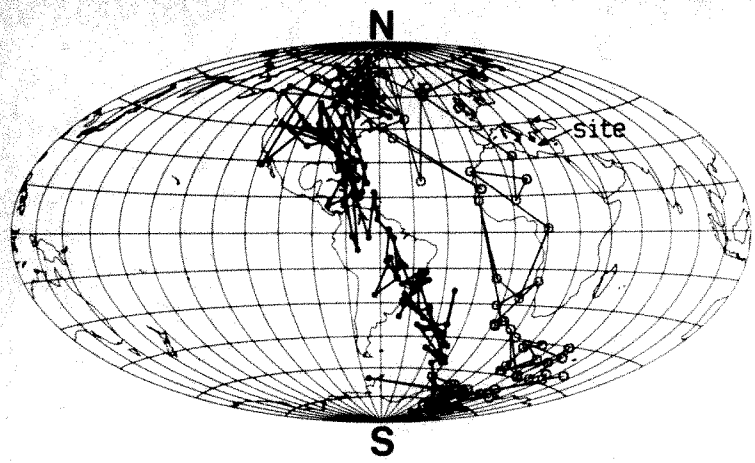


Fig. 2 Paths of virtual geomagnetic poles associated with the KS06 (solid symbols) and KP02 (open symbols) transitions. For KS06 the path depicts north-VGP, for KP02 south VGPs. The two paths appear to be clearly distinct, although an east-to-west migration of intermediate VGPs exists in both of them.

but in our opinion still significant when the old N–R record is used). Moreover, the following remarks can be made.

As pointed out by Hoffman, an east-to-west migration of intermediate VGPs is a common feature of all three paths. In our paper[3], we had erroneously thought that this characteristic was related to properties of the Skouloudhiana section. We now agree with Hoffman that it is related to the reversal's mechanism.

The N–R transition in Potamida lies outside the time interval between the two R–N reversals in Skouloudhiana, so the difference observed in the VGP paths indicates that some differences exist between the transitional field configurations relative to KS02 and KS06 on one side and KP02 on the other. It does not, however, yield any information about the N–R transitions occurring during the time interval between KS02 and KS06. Data about these transitions are thus needed to determine whether the differences observed in the VGPs paths are due to some changes in the reversal mechanism occurring between KS06 and KP02 or whether transitions of opposite sense are always associated with different field configurations.

In Skouloudhiana, unfortunately, the NRM intensity of the sediments at the corresponding stratigraphic horizons is very low, so that measurements are difficult. However, some measurements are now in progress using a cryogenic magnetometer, and we hope to solve this problem.

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Factors determining desert dune type

IN their study of aeolian dunes, Wasson and Hyde[1] concluded that sand supply is an important determinant of dune type. Their study was based on the assumption that equivalent sand thickness (EST, defined as the ratio of sand volume within dunes to the area of a dune field) can be used to quantify sand supply and sand availability. Our purpose here is to argue that EST is a measure of dune size and shape, not a measure of sand supply. As a result, the technique of equating EST and sand supply can be shown to lead to incorrect conclusions when tested with subaqueous bedforms.

Sand supply and sand availability are general terms that might be loosely used for at least five quantities: (1) the volume of sand within dunes in a unit area of dune field (equal to EST), (2) the total volume of potentially erodable sand in a unit area of dune field, including sand below the surface that passes through dune troughs, (3) the fraction of a dune field covered by erodible sand, (4) the rate at which sand is supplied to a unit width of dune field from upwind sources, and (5) the rate at which sand accumulates in a dune field (that is, the rate of deposition or erosion, which is equal to the difference between the rates at which sand enters and leaves a unit area of dune field[2]). The first two quantities have units of length, the third is dimensionless, the fourth has units of area/time, and the fifth has units of length/time. In precise usage, the parameters that measure sand abundance or availability [quantities (1)–(3) above] must be distinguished from the parameters that measure rates of sand transport [quantity (4)] or accumulation [quantity (5)].

Wasson and Hyde state that 'Sand supply and EST are often identical but the possibility of sand moving through dunes into areas outside dune fields ... means that EST and sand supply may not be exactly the same for highly mobile dune fields, in areas of high wind energy.' Although no single parameter can quantify all five types of sand 'supply' listed above, the statement quoted seems to imply that a quantity measured in units of length (EST) might somehow describe a sediment-transport-rate quantity if the rate is not too high.

Another problem with using EST to measure sand availability is that EST does not include erodible sand that may occur below the surface that passes through dune troughs—a quantity that may represent the bulk of sand in a dune field. In other words, EST is a measure of available sand only if all available sand in a dune field is incorporated within dunes. This assumption, which forms the basis for Wasson and Hyde's analysis, is unsupported, and it is clearly incorrect in areas where dunes deposit cross-stratified beds, because in such areas of deposition, sand is transferred from dunes to underlying beds[2]. The problem of quantifying sand availability might be eliminated by measuring quantity (3) listed above (the fraction of a dune field covered by erodible sand), rather than measuring EST.

Because EST is independent of fluid medium, it is instructive to test Wasson and Hyde's analytical technique with subaqueous bedforms, about which more is known. From a plot of subaqueous bedforms as a function of EST and current variability (like Wasson and Hyde's Fig. 4) one might incorrectly conclude that sand waves occur where sand is abundant, that upper-regime flat beds occur where sand is scarce, and that ripples occur where sand abundance is intermediate. (In reality, flow strength is a more important determinant of what type of subaqueous bedform is produced.) The point of considering subaqueous bedforms is not to argue that aeolian bedforms behave similarly, but rather to demonstrate that, regardless of fluid medium, a correlation between bedform type and EST does not prove that EST (or sand supply) is a determinant of bedform type. Instead, EST may merely reflect bedform type, with bedform type determined by other parameters.

As a result of their EST-based analysis, Wasson and Hyde conclude that; (1) barchans occur where there is very little sand, (2) transverse dunes occur where sand is abundant, (3) longitudinal dunes occur where there is little sand, and (4) star dunes occur where sand is abundant. Conclusion (1) is undoubtedly correct, but the other three conclusions are suspect. For example, transverse dunes form in some areas with so little sand cover that underlying rocks or sediments are exposed in deeper parts of dune troughs, and the dune height in some such areas is so small that the mean thickness of erodible sand in the dune field is no more than a few metres.

The conclusion that longitudinal dunes occur where there is little sand is unjustified because the EST measurements on which that conclusion is based are incapable of identifying areas with little available sand (because additional sand may underlie dune troughs).

This question about whether longitudinal dunes can occur where sand is abundant has important ramifications to geologists studying aeolian deposits. If longitudinal dunes can occur only where sand is scarce, they should be unable to produce deposits without causing their own destruction or evolution into other kinds of dunes.

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WASSON AND HYDE REPLY—The aeolian sand both in dunes and swales is that proportion of the total arenaceous sediment, lying either beneath or upwind of a dune field, that has been made available for deflation and dune construction. It follows that equivalent sand thickness (EST) in a dune field in which all of the aeolian sand lies within the dunes is a good measure of the sand made available for dune construction. In dune fields where a large volume of aeolian sand lies in swales, EST is a minimum estimate of the total volume of aeolian sand in the dune field. We do not claim[1], as Rubin believes we do, that EST equals the total arenaceous sediment body that might be deflated in either conditions different from those that have prevailed during the history of the dune field or at some future time. Observations in a number of dune fields clearly show that not all sandy sediments have been deflated to form dunes. In the Thar, Simpson and Kalahari, for example, large stores of non-aeolian sand lie both beneath and upwind of the dunes[2,3,4].

Examination of pits, wells and bore-logs both by us and by others shows that significant quantities of aeolian sand lie in swales in fields of star and transverse dunes, very little in fields of longitudinal dunes and none in areas of barchans[5]. The inclusion in estimates of EST of this fraction of aeolian sand would therefore enhance the differences between star/transverse and longitudinal/barchan dunes on our Fig. 4.

We concluded that drift potential (DP, a measure of wind strength) does not correlate with dune type but we now go on to suggest that vegetation and sand moisture have always been modulators of sand transport rates in presently arid to semi-arid dunefields (for example, Australia, Thar, Kalahari). In presently hyper-arid dune fields (Namib, Sahara, Rub'al Khali) sand transport has always responded directly to the fluid medium. We also note the possibility that EST has been controlled by a number of meteorological and biological factors that differ both between dune fields and through time. The control of subaqueous bedforms by the fluid medium does not have a simple parallel in subaerial dune fields. For example, there is no equivalent of vegetation in river bedforms, and the density differences between solids and the transporting media in the two cases cautions against a simple comparison of the kind suggested by Rubin. Therefore, a test of our ideas about desert dune fields using subaqueous bedforms is casuistry. Rubin notes that he does not wish to infer that aeolian and subaqueous bedforms behave similarly, making his comparison even more puzzling.

One of Rubin's objections to our conclusions is his observation that transverse dunes form in some areas where swales are not covered by aeolian sand, thereby implying low EST. In our Fig. 4 only 1σ is plotted about the mean of EST for each dune type so, clearly, low values of EST were included in our sample. We do not claim that all values of EST in transverse dune fields are higher than all values of EST in longitudinal dune fields. We have used statistical significance as our criterion of difference. Rubin's citing of a few undocumented and unquantified examples unfortunately follows in a long tradition in aeolian geomorphology. We specifically set out to quantify relationships using all of the best data available to us, so as to avoid the selective use of examples.

It is relatively easy to see how the variable RDP/DP might influence dune type. The role of EST is less clear, and so our use of this variable is best viewed as an aid to discrimination between dune types. If transport rates have always been higher in presently hyper-arid dune fields, by comparison with presently more humid dune fields, then it is possible that EST reflects, among other things, the presence or absence of long-distance sediment transport by wind. Star dunes have the highest values of EST and occur in areas in the Sahara where sand flow converges[5–8]. Simple longitudinal dunes in the Simpson–Strzelecki dune field of Australia have low values of EST and have been derived from sediment sources close by[2]. It is conceivable that EST reflects dune type rather than vice versa. Rubin's comments have highlighted this unresolved problem but we cannot agree with his conclusions.

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J. A. MABBUTT COMMENTS—The exchange of views between Wasson and Hyde[1] and Rubin has been useful in clarifying controls over sand availability for dune formation, but in my view does not go far enough in considering how this in turn acts to determine major dune type. This determinant would appear to be the extent of non-dune surface suited to the rapid transmission of saltating sand, relative to the area of sand-trapping dune surface. Where the former is so dominant as to leave the dune accumulations isolated, barchans characteristically develop; where the relationship is such as to allow the continued exposure of downwind-oriented non-dune strips, longitudinal dunes are typical; where, on the other hand, the non-dune floor remains completely buried or revealed only in minor and non-connecting troughs, transverse dunes are found. In many dune fields these relationships occur in sequence downwind towards a basin depression of sand accumulation, and there is a corresponding downwind sequence of dune form. A whole range of circumstances can intervene to determine these relationships locally, including thickness of prior sands available for wind-working into dunes, current aeolian sand supply, the transmissivity of the underlying floor, wind energy and vegetation cover.

Patterns of longitudinal dunes, in which dune and non-dune surfaces are more evenly matched in extent than in the other dune types, are strongly influenced by variations in the nature of this non-dune surface. A recent account of longitudinal dunes in the north-west Simpson Desert[2] shows that the non-dune 'floor' can vary locally between soft unconsolidated sand (partly wind-worked sandy alluvium?), firm surfaces of coarse-textured alluvium, and hard gibber pavements. The dunes that cross these surfaces may accordingly vary between wind-sculpted forms, sand mounds assembled *in situ*, and transgressive dunes. There is strong evidence that differences in sand-transmissivity between the non-dune 'floors' have contributed to differences in spacing and other pattern attributes.

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Singular mathematics

n Goodwin

Catastrophe Theory. By V.I. Arnold. Translated by R.K. Thomas. *Springer-Verlag: 1984. Pp.78. Pbk DM 16.80, $8.50.*
Mathematical Models of Morphogenesis. By René Thom. *Ellis Horwood/Halsted: 1983. Pp.305. £25, $59.95.*

"THE nice results of singularity theory are happily not dependent upon the dark mystics of catastrophe theory". Thus V. I. Arnold, a distinguished Russian mathematician, sums up a widely-held professional view of the most extraordinary mathematical development of the century. However, in René Thom's new book, a collection of essays written between 1967 and 1981, Thom goes a long way towards clarifying some of the misunderstandings that lie behind Arnold's judgement, which reflects certain deeply-held prejudices of the scientific community. To put this far-reaching conflict of views into perspective, the remarkable history of catastrophe theory needs to be considered.

Ten years ago, an area of deep and difficult mathematics, involving profound theorems that had aroused the interest of the world's finest mathematicians, suddenly became the topic of newspaper and magazine articles, common-room discussion and party gossip. This resulted from two events: the publication in 1974 of René Thom's first book, *Structural Stability and Morphogenesis*; and E. C. Zeeman's communication to the International Congress of Mathematics in Vancouver in the same year, describing the application of Thom's ideas to a great variety of problems, not only in mechanics and physics but also in biology and the human sciences.

Thom had extended and developed a theory of the mathematician H. Whitney, published in 1955. Whereas classical (i.e. Newtonian) differential calculus considers only smooth, continuous processes, Whitney's theory allowed for a systematic study of the discontinuities, breaks or jumps that can occur in a dynamical process, described by its singularities. Smooth mappings and their singularities are extremely widespread and familiar: flows and wave formation in liquids, meteorological fronts and turbulence, regions of intense illumination due to light (rainbows, curves of bright light at the bottom of swimming pools), the condensation of water vapour, the formation and propagation of defects in crystals, and so on. Following Whitney's basic study, singularity theory developed very rapidly and acted as a focal point for the combination of some of the most abstract areas of mathematics (differential and algebraic geometry and topology, group theory, commutative algebra etc.) with some of the most applied (stability theory, bifurcation theory, geometrical optics etc.)

Thom combined the abstract and the concrete to formulate a comprehensive taxonomy of the singularities that could occur in spaces of four dimensions — three of space and one of time; the world as we know it — suggesting that these singularities are the origins of all natural forms that arise in a sufficiently structured (e.g. polarized) system. Christopher Zeeman located this theory within the broader context of general systems, greatly extending the potential applications of the theory and including the human and social sciences; and he it was who suggested the term "catastrophe theory". The dual offer of a systematic theory describing the genesis of form from Thom, together with Zeeman's examples illustrating its non-linear dynamical applications, suddenly opened a door to an extraordinary world of mathematical modelling. Large numbers of people passed through, but soon many were shouting that they had been offered false goods; that the emperor had no clothes. What did they find in that promised land?

Arnold's clear and often entertaining little book (for which the translator should take some credit) casts a caustically illuminating eye over what he sees as a typically Western marketing phenomenon, summing it up in a quotation from Dorochevich: "I think, my dearest, that all this decadence is nothing more than simply a way to approach tradesmen". His own scientific judgement is severe: "In the majority of serious applications, the singularity is a Whitney cusp [describing basic reversible discontinuities or phase transitions, such as gas condensation and vaporization] and the result was known before the advent of catastrophe theory". This is certainly an over-reaction, for there are thoroughly rigorous applications of the higher-order catastrophes to physical phenomena that depend upon Thom's extension of the Whitney theory, such as the use of the elliptic umbilic to study and *predict* hydrodynamic stagnation points in the six-roll mill experiments of Berry and Macklin. And, in biology, Zeeman has applied catastrophe theory in original and useful ways to morphogenetic processes in embryos, making predictions and stimulating experiments the results of which are consistent with the model. However, Thom distances himself from such applications, insisting that there is a much more profound use of catastrophe theory than the rigorous quantitative analysis that Arnold takes to be the measure of mathematical modelling and good science. What is this vision?

The subtitle of Thom's first book was *An Outline of a General Theory of Models*. Despite its title, this second book is, like the first, more about a general theory of modelling than about actual models. Readers may or may not like this style of theorizing, but once accepted the book can be read for what it is: an inspired and imaginative exposition of both a scientific methodology and a research programme, illustrated in diverse contexts (physics, biology, psychology) by using catastrophe theory to sharpen questions rather than to obtain answers to specific problems. Like a piece of classical theatre, the climax of the book comes in the middle, Chapters 7 and 8, containing Thom's defence of his conception of his theory. With a gesture characteristic of the central image of catastrophe theory, the bifurcation set of the cusp, Thom opens our imaginations with a perceptive discussion of the aims of science and the nature of space-time, magic and geometry, and then brings these perennial themes into sharp focus on a problem for which he offers, not an answer, but a programme of investigation.

The discussion is centred on the struggle to unify action with understanding, the former aiming to resolve local problems while the latter seeks the universal or the global. Thom argues that the great scientific successes (Newtonian gravitation, electromagnetism, quantum mechanics, relativity theory) are non-local theories that describe the magic of action-at-a-distance in terms of strictly controlled local manifestations: for example, Maxwell's equations localize electromagnetism; Einstein rendered gravitation local. Theories that have no "magical" non-local elements, argues Thom, do not introduce new possibilities of action. "Catastrophe theory, anti-magic *par excellence*, pushes the principle of locality to the extreme: this is without doubt why it hardly offers new practical possibilities". Must it not, then, also fail to provide insight or understanding, since it has no global properties? Here Thom offers a research programme the objective of which is to construct the global from the local by a procedure in which the *Urbild* of spatiality, the generative principle of space, is an organizing centre that unfolds, like the big bang model in cosmology or the embryo which develops from the egg. This is clearly congenial to catastrophe theory which is precisely about the unfolding of form from organizing germs in space-time, about those singularities which contain in intensely localized form the potential for generating a specific, extended structure. But a rigorous mathematical theory of such a generative procedure is as yet non-existent.

The absence of an appropriate theory, however, does not stop Thom from exploring implications for research in biology,

linguistics, semiotics and semantics. His cognitive programme is to geometrize thought itself, using the elementary catastrophes as logoi, the algebraico-geometrical structures which stabilize concepts in mental activity space. Semantics is based upon approximate isomorphisms between logoi of material beings and those of the corresponding concepts, and so mind perceives real properties of objects which are the "bearers of meaning". His objective is then "to create a theory of meaning whose nature is such that the act itself of knowing is a consequence of the theory; i.e., when an appearance appears to us as a bearer of meaning, we will know why and to what formal facts to attribute it".

What people have primarily reacted against in Thom's writing is the highly discursive style combined with the sharply-defined analytical tools of abstract mathematics. We are used to having mathematics applied to specific problems, leading to solutions. Thom applies it to perennial philosophical and scientific problems, leading to further questions. Clearly catastrophe theory works in physics, where singularity theory worked before, and it is clear how to apply it following that lead. This is the path of wisdom Arnold recommends and illustrates. No one has yet shown how to apply catastrophe theory to the general problems of morphogenesis, cognition and the new physics in the style of a generative science of form suggested by Thom. Until it is tried, it seems premature to condemn it — but no one should be in any doubt about the difficulty of such a programme. □

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Thin-necked upright display posture of the male black-billed capercaillie. The drawing is reproduced from Paul A. Johnsgard's *The Grouse of the World*, published by the University of Nebraska Press in the United States and Croom Helm in Britain. Price is $42.50, £30.

Sense of energy

Ian Smart

Sun Traps. By John Elkington.
Penguin/Viking: 1984. Pp.400.
Pbk £3.95, $6.95.

EMBEDDED in Mr Elkington's chapters, on fossil fuels and nuclear power as well as renewable energy sources, there is a feast of fact, from a photovoltaic hat to the mass of firewood needed to dry a kilogram of ginger. Like many "popular" energy books, this one tries to handle an astonishing range of topics by assembling anecdotes about all of them, larded with numbers and speckled with names. It is described as a "comprehensive survey on solar power and future energy". But honest efforts to follow the thread of argument through the labyrinth are repeatedly foiled: diverted into yet another garden of colourful trivialities, or distracted by dramatic writing in the "little-did-he-know-that" tradition.

None of this prevents Mr Elkington from being informative. Nor, which is more important, does it hide his good sense — although it sometimes overshadows it. He begins by pointing out that technical feasibility is no substitute for — or index to — economic competitiveness. And he ends with a studiedly moderate estimate of the contribution renewable sources will make to world energy supply in any of our lifetimes. His assessment could only have been reinforced by more persistent attention to economics. As it is, its moderation serves to highlight the euphoric exaggeration by so many of the sun, wind and wave enthusiasts from whom he quotes: a habit of hyperbole matching the former excesses of nuclear apostles and inviting a similar fate.

Mr Elkington himself gets the balance about right, by characterizing as only slightly optimistic a Worldwatch Institute projection that the renewable contribution to global energy supply will rise from 16.5% in 1980 to 23.9% in 2000 — with three-quarters of the increase coming from hydroelectricity, geothermal power and fuelwood. He is no less right to insist that solar-based energy, exploited at reasonable cost and applied in appropriate ways, must make a larger contribution to human welfare. Unfortunately, he barely touches on the fundamental problems involved: the political and economic tension between centralized renewable energy systems, using "high" technology, and decentralized networks, seeking social as well as technical simplicity; the need to choose between ecologically incompatible interests (as between nature conservation and biomass development); and the ultimate dilemma of whether energy should be converted to accommodate society's demands, or social preferences adapted to efficient energy conversion. It is a pity he did not sacrifice more of his anecdotal scrapbook to exploring them. For, through these thickets of lore, there shine rays of a judicious realism. □

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Walk in the gardens

George Oster

An Introduction to the Physical Chemistry of Biological Organization.
By A.R. Peacocke.
Clarendon: 1983. Pp.302. £35, $65.

FORMALISMS are seductive sirens, for they seem to unify so many disparate threads and they promise deep insights using only mathematical manipulations. Of these seductresses, surely thermodynamics is the most alluring. One of its foremost practitioners, the late Aharon Katchalsky, once observed wryly that "it is such a marvelous tool: you don't know what you put in, and you don't understand what you get out. But you know it must be right!".

In physics and chemistry, thermodynamics has performed as advertised; however, in biology it has yet to live up to its billing. By now, no one expects living systems to disobey the laws of thermodynamics, but these tenets are crude and vague constraints when applied to such systems, and they provide very little insight into the operation of biological entities. Nonequilibrium thermodynamics, by extending the venue of classical thermostatics into the realm of dynamics, promised to deliver new understanding and unifying concepts which could shed light on the mysteries of biological organization. But has it? Well, that is a matter of dispute, and reasonable scientists can disagree — although on this subject they usually do so with righteous zeal.

Clearly, Professor Peacocke feels that the sirens' song is worth pursuing, for he devotes the first half of his book to the subject of "Thermodynamic Interpretations of Living Systems". This label is a bit misleading, for discussion of living systems is rarely opened. Only in a few pages of these chapters is any application to specific biological problems attempted, and these attempts consist largely of recasting several biochemical models in the language of network thermodynamics. Beyond a certain notational and conceptual simplification — not to be sneezed at, to be sure — it is not clear that any new understanding emerges.

Chapter 1 commences with a discursive and philosophical excursion into the muddy waters of "complexity" and "hierarchies", finally enunciating the "central problem of biology" as (according to H. Pattee): ". . . the origin and operation of

the hierarchical constraints which harness matter to perform coherent functions". Well, it is true that we would like to know how things work, but recasting the problem in social science lingo doesn't enlighten me. Indeed, Professor Peacocke has collected together a virtual thesaurus of quotations which he employs to illustrate and entertain as he leads us in Chapters 2 and 3 through the various formalisms of irreversible thermodynamics developed by the disciples of Prigogine and of Katchalsky. And an unusually clear tour it is, although somewhat sparse in mathematical detail.

Part II of the book is entitled "Kinetic Interpretations of Living Systems". Here there is more contact with specific situations, and less development of formalisms — perhaps because the subject matter is more inherently mathematical. However, the applications are more chemical than biological, and the author's treatment barely ripples the surface of some quite deep mathematical waters. For example, in Chapter 4, "The Kinetics of Biological Organization", he describes various chemical schemes which, when combined with diffusion, can produce spatial concentration patterns. He mentions the best work in this field, notably that of Meinhardt and Murray, but the treatment is mostly descriptive, albeit lucid. It seems to me that Professor Peacocke frequently opted for a philosophical digression rather than a detailed description of how to perform the calculations required to test whether a model can exhibit spatial instabilities. These recipes are admittedly complicated, but their absence points out the major problem with the book: it is too sketchy on detail to serve as a textbook, but too mathematical to provide biologists with an introduction to a number of active research areas in physical chemistry. However, I found the book easy and pleasant reading and the wealth of references to be most useful. Chapter 5, which describes theories of macromolecular evolution, especially that of M. Eigen, is especially clear. The concluding chapter, "The Interpretation of Biological Complexity", is, like the introduction, a collection of philosophical quotations from various authors on how to best tackle the problems of biological organization.

I am finally left with the feeling that I was glad to have read Professor Peacocke's book; but I am not sure what the wider audience for it will be. Biologists will not find that the mathematics leads them to new experiments, and physical chemists will have to consult more detailed treatments to acquire the techniques; but both may find some agreeable perspectives from which to view their work. Perhaps the book should be read for what it is: a philosophical walk through the gardens that Professor Peacocke finds most beautiful. □

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Poet in the field

Richard Mabey

The Natural History Prose Writing of John Clare.
Edited by Margaret Grainger.
Clarendon: 1984. Pp.397. £35, $67.

THE STANDING of the agricultural labourer-turned-poet, John Clare (1793–1864), as a nature writer has never been higher. Yet his reputation as a *scientific* naturalist has until now rested on his adding to the Northamptonshire lists 65 first county records for birds and more than 40 for plants. These figures are the more remarkable when you consider that they were worked out retrospectively from his poems, and are backed up by nothing more substantial than the absolute conviction his poetry carries of being based on personal experience. Poets might be inclined towards invention, but not, so Clare mythology insists, one who was also a peasant.

Now, thanks to an impressive and sympathetically edited work of scholarship by Margaret Grainger, we have access to an apparently more objective record of Clare's field observations. Mining the seemingly inexhaustible supply of old notebooks, letters, scribbled envelopes and book jackets in the Clare manuscript collection in Peterborough Museum, Ms Grainger has pieced together the bulk of his natural history prose written between 1820 and 1850. Some is little more than random jottings, but there are also the first notes towards a planned *Natural History of Helpstone* (he had read Gilbert White) which he planned to call *Biographys of Birds & Flowers.*

I said "apparently more objective" above because the first thing that strikes you about this collection is how adept this man, supposedly blessed or imprisoned by an "innocent eye", was at *choosing* how he wrote, and at moving between scientific and vernacular modes of classification and expression. In a detailed letter to the publisher Hessey about Elizabeth Dent's *Flora Domestica,* for instance, he writes:

> *Cardamine* page 74 this is calld lilac with us as well as 'ladysmock' but I never heard it calld cuckoo in my life otherwise then [*sic*] by books — the wood anemone is also calld 'lady smock' by childern what the common people call 'cuckoo' with us is one which is a species of the 'Orchis'. . . .

This upstart and professional tone was not at all what his publishers wanted from their tame ploughboy, and it is no surprise that his proposed *Natural History* never came to fruition.

But his working journal (kept between 1824 and 1828) does survive, and is perhaps the most fascinating part of this book — not so much because of the accuracy and rapt attention of his descriptions, but because, just as in the poems, they are worked without any change of perspective into accounts of his own feelings. The life of the fields and of Clare's imagination are one and the same. He saw himself, crucially, as *part* of nature. On 10 September 1824, he is feeling unwell, and records a sorrowful note on the swallows congregating in the walnut trees and chattering as "if they were telling their young stories of their long journey to cheer & check fears". The next day he is writing an essay on "the sexual system of plants" for his *Natural History,* and reading a review of Goethe in the *London Magazine.* And in the journal's appendix there is an



Mansell Collection

John Clare: *part* of nature.

electric account of a meeting with a "will o whisp":

> it blazd out like a whisp of straw & made a crackling noise like straw burning which soon convinced me of its visit the luminous haloo that spread from it was of a mysterious terrific hue & the enlargd size & whiteness of my own hands frit me the rushes appeard to have grown up as large & tall as walebone whips & the bushes seemd to be climbing the sky. . . .

These extracts are classic Clare, troubled, sensitive, highly literate despite the idiosyncratic grammar. Ms Grainger has done for the prose what John Barrell did some years ago for the poetry, demonstrating that its style and perspective are as much a consequence of Clare being jerked "out of his knowledge" by self-education and the disorientating effects of enclosure, as of the depth of his local roots. And in so doing her book raises questions about whether there is any essential difference between "subjective" and "objective" knowledge, and for that matter between poetic and scientific description. Towards the end of the book is Clare's list of the orchids he knew from Helpston parish (16 species, remarkably). In the precise and affectionate detailing of the places in which each species grew — early marsh orchid was plentiful "on a spot called Parkers Moor near Peasfield-hedge & on Deadmoor near Sheef green . . . but these places are now all under plough" — it is both a haunting evocation of the inner geography of Clare's experience, and the most exact account of locations that any historical botanist could demand. □

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Ice flow and applied mathematics

Charles F. Raymond

Theoretical Glaciology.
By Kolumban Hutter.
Reidel/MTP Press: 1983. Pp.510.
Dfl. 240, $104, £60.95.

THE modern phase of glaciology opened in the late 1940s when a small contingent of physicists became interested in the motion of glaciers and ice sheets. Typical of physicists, they emphasized a research method which isolates characteristic parts of glacier behaviour to illuminate essential physical relationships, and they initiated a progressive growth in understanding that continues today. In the past few years a group with a strong applied mathematical bent has come upon the scene. These scientists like to state problems in their fullest mathematical generality, simplifying them only to the extent needed to make a solution feasible. They bring a new analytical expertise to glaciology.

Although the physical and the applied mathematical approaches are essential complements to one another, in the current glaciological community there is something of a communication gap between people who identify with one or the other. Kolumban Hutter is identified firmly with the applied mathematical viewpoint and through this book advocates it. However, Hutter also recognizes the communication gap and tries to bridge it. He has succeeded in writing a unique and rich synthesis that will please any glaciologist who makes the effort to delve into it.

In broad plan the book proceeds from a brief development of general concepts of continuum physics (Part I), to their application to specific problems in the flow of glaciers and ice sheets (Part II). Along the way there is a narrowing of complexity in the physics and a step-by-step broadening of complexity in geometry and time dependence of boundary conditions.

Part I (about one quarter of the book) covers balance laws for mass, momentum, energy and entropy, the continuity (or jump) conditions for these quantities at boundaries, and constraints on the form of constitutive relations. These principles provide the rigorous framework for describing realistic mechanical and thermodynamic behaviour of both polar (sub-freezing) and temperate (wet) ice.

Part II deals with ice motion in geometries of increasing complexity, starting with a parallel-sided slab and progressing to three-dimensional problems. In addition, the problem of determining the geometry of the free upper surface under steady or time-varying climate is examined. While these topics have been considered in earlier books and review articles, Hutter's development is dramatically different and more expansive. Although a number of mathematical techniques are employed, perturbation expansions are a recurring theme. These analyses impressively demonstrate how far one can proceed with analytical methods, despite non-linearity of ice creep, a basal sliding law, and coupled mass and heat flow.

In the second part in particular, Hutter writes with clarity and confidence. Each chapter is crafted to stand independently yet fit into the whole. The balance of text and equations enables a reader to follow the thread of the mathematical development without necessarily studying all of the manipulative details.

Theoretical Glaciology does not attempt to provide much information about real glaciers or ice sheets. It also does not cover the physics of glacier sliding or water motion through glaciers, which are topics of great importance and considerable research activity. By itself, then, it is not suitable as a text for a general course in glaciology. However, for a graduate-level theoretical course in glacier dynamics it is excellent, and any glaciologist working in glacier dynamics will find access to the book to be essential. Although it remains to be seen whether *Theoretical Glaciology* will open a new era of glaciological research, it will undoubtedly set a new standard for mathematical analysis of ice flow. □

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New in paperback

- *The Myths of Human Evolution* by Niles Eldredge and Ian Tattersall. Publisher is Columbia University Press, price is $10.95 (in the United States and elsewhere). For review see *Nature* **303**, 90; 1983.
- *Microman: Living and Growing with Computers* by Gordon Pask and Susan Curran. Published by Century, London, price £4.95. For review see *Nature* **302**, 771; 1983.

Taxonomy of vision

David J. Tolhurst

Parallel Processing in the Visual System: The Classification of Retinal Ganglion Cells and its Impact on the Neurobiology of Vision.
By Jonathan Stone.
Plenum: 1983. Pp.438. $55, £42.35.

THE mammalian retina contains several populations of ganglion cells. Each population extracts information about different aspects of the visual world and conveys that information differentially to the brain. The notion of parallel processing of visual information has become so pervasive that anatomists, physiologists and psychophysicists are increasingly tempted to explain any observation in such terms. There is now a large mass of data which needs critical examination, partly to distinguish the real from the spurious and partly to resolve the many discrepancies.

It is disappointing, therefore, that Jonathan Stone has not chosen to provide a straightforward review of that published data. Rather, he uses the literature as a vehicle for expressing his views that the classification of ganglion cells is in itself the primary goal and that the central processing of visual information can be understood only in terms of parallel processing. There is no systematic description of the several populations of ganglion cell, and the references to the literature are often so cryptic that arguments cannot be followed unless the reader has a good knowledge of what has been written on the subject. It is unclear why some observations are discussed at length whilst others are ignored. The book, then, is not an introduction to the subject.

Stone's first objective is to classify ganglion cells. He argues with some merit that the taxa should be based on an assessment of the cells' overall behaviour rather than on one "key" feature of behaviour. However, he does not explain how one should classify a cell when individual aspects of its behaviour seem to conflict. The first climax of the book is the proposal that X and Y cell classes are not at the same taxonomic level as the W cell class. Rather, X and Y cells are subclasses of a single XY class. The questions of why there are two kinds of cell and why their behaviour differs in particular ways is hardly considered.

The notion of parallel processing has certainly had an impact on the neurobiology of vision: there are many papers that mention it. However, Stone does not make clear how visual science would have floundered without the invocation of X, Y and W cells. The central processing of visual information is described very firmly from the contention that there must be parallel processing of the information conveyed by X, Y and W cells. Deviations from the theme are dismissed. For instance, the X/Y/W classification is orthogonal to Hubel and Wiesel's classification of simple and complex cells in the visual cortex. Stone implies that the simple/complex classification must, therefore, be unsatisfactory and concludes: "the question of the organization of the visual cortex to process the activities of different groups of ganglion cells is being successfully pursued without employing the 'simple'/'complex' distinction among cortical cells".

Perhaps Stone's use of quotation marks in describing simple and complex cells reveals the degree of objectivity in his discussion of parallel processing in the visual system. This is a book for the converted. □

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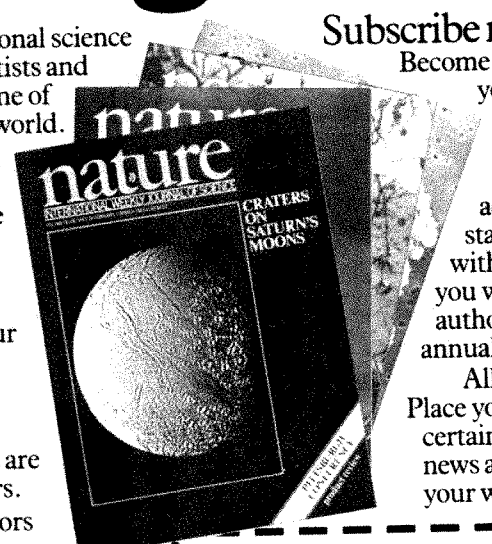
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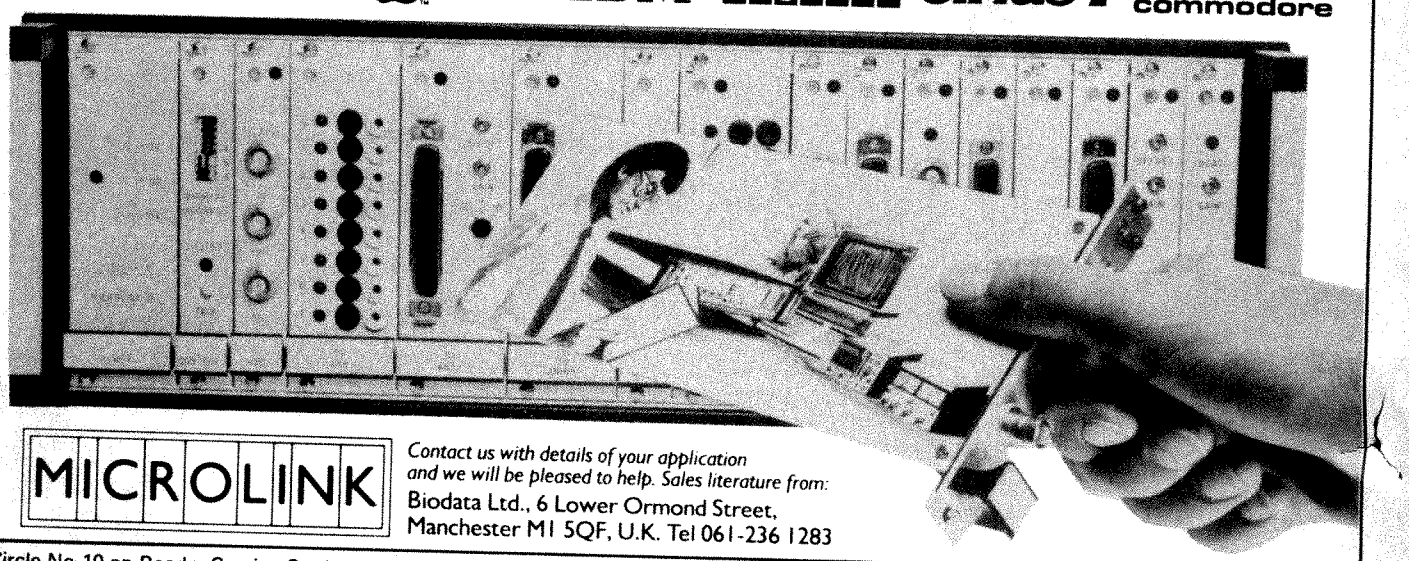
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Circle No.03 on Reader Service Card.

Laboratory apparatus calling

Eleven new products are described this month including a device that "phones home" when your experiment goes wrong.

● The Accuspec "second generation" compact gas chromatograph measures only 12½" × 12½" × 15½" high yet offers research-grade performance and a full range of features. The full dual column oven provides a choice of three capillary systems or ⅛" packed column facilities. Automatic cooldown is standard with the temperature programmer. Dual FID, micro-TCD and specific detectors are available.
Circle No. 140 on Reader Service Card.

● The Laser Analytics Division of Spectra-Physics Inc. has developed a cleaved-coupled-cavity Pb-salt diode laser that operates single mode over limited current and temperature ranges. The new device consists of two coupled cavity sections: the front section performs as an emitting laser and the rear section as a modulator. By varying the modulator current, it is possible to tune the emission from the front section thereby obtaining additional precise control of the laser emission characteristics.
Circle No. 141 on Reader Service Card.

● Perkin-Elmer's LC-15B is a new, low-cost, fixed wavelength UV detector for liquid chromatography. At the standard 254-nm wavelength, the maximum noise is $\pm 1 \times 10^{-5}$ absorbancy units while drift is less than 2×10^{-4} absorbance units. Range settings down to around 0.0001 AUFS are included, with response times of 0.2, 0.5 and 1.0. Other features of the LC-15B include a push-button event mark, large digital readout and wide zero baseline adjustment range.
Circle No. 142 on Reader Service Card.

● A high sensitivity auto-ranging electrostatic 'fieldmeter' has recently been developed by John Chubb Instrumentation of Cheltenham. The JCL 101 gives stable measurement of electric fields in the vicinity of 'static' charges on insulating or conducting surfaces.
Circle No. 143 on Reader Service Card.

● Agarose IEF is a highly purified agarose optimized for isoelectric focusing by charge balancing. Gradient drift is reduced to a minimum to give high resolution, stable *p*H gradients and efficient isoelectric focusing. Agarose IEF is produced by Pharmacia.
Circle No. 144 on Reader Service Card.

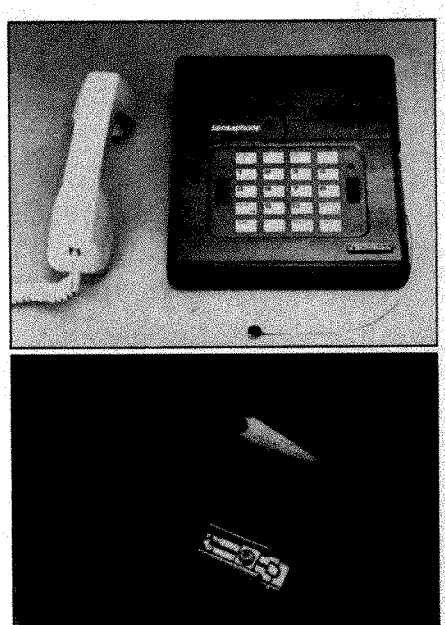
These notes are based on information provided by the manufacturers. For further details circle the numbers on the Reader Service Card bound inside the journal.

● *Ulex europaeus* agglutinin I (UEA I), a plant lectin recently claimed to be superior to antibody to factor VIII as an endothelial cell marker, is available from Vector Laboratories. UEA I has been shown to identify vascular endothelia in kidney, liver, pancreas, lung, skin, brain, placenta, umbilical cord and cultured cells as well as various tumours of endothelial origin. Unlike anti-factor VIII antibodies, UEA I can be used equally well on snap frozen or paraffin embedded tissues. Several detection systems are available including fluorescein or rhodamine conjugates for fluorescent microscopy, and affinity purified antibodies to UEA I or biotin-labelled UEA I for biotin/avidin-based histochemical techniques.
Circle No. 145 on Reader Service Card.

● The Thermex interface from Columbus Instruments converts an Apple II Plus or Apple IIe computer into an automatic printing 16-channel thermometer. As sensor, it utilizes J, K or T thermocouples. Resolution in the biological temperature range is 0.1 °C. In other temperature ranges accuracy of 0.2% full-scale is achieved by individually calibrated thermocouples. A special calibration program is available to allow the user to calibrate and linearize thermocouple probes in any desired range. Thermex has built in cold junction compensation. Biological probes are available for rectal, intravenous and implantable applications for use in animals. A video monitor displays current temperatures in all channels.
Circle No. 146 on Reader Service Card.

● An ultra-stable stirrer speed in the range 0–1,250 r.p.m. is possible with the ADI 1012 stirrer control unit. Manufactured by Applikon B.V., the controller is specially suited for use in fermentation vessels. With the aid of the ADI 1012's remote speed control input, stirrer speed can be regulated automatically with respect to the culture's oxygen content.
Circle No. 147 on Reader Service Card.

● Antibodies for the detection of oncogene protein products and related antigens — such as the epidermal growth factor receptor — are now available from Oncor. The antibodies are intended for use in immunofluorescence, immunoprecipitation and enzyme-linked assays. The range consists of: AB*myc*.1, AB*ras*.1, AB*src*.1 and AB EGF-R.1. Each antibody is supplied with sufficient product to perform 50 RIP-PAGE assays.
Circle No. 148 on Reader Service Card.



The Omega laboratory monitor (top) and Spectra-Physics new laser

●Constant process monitoring is possible with the new Omega LM-1000 laboratory monitor with voice simulator. It monitors a wide variety of process and laboratory conditions via a standard telephone jack. The LM1000 will monitor conditions such as: room air temperature or ambient sound levels — the LM-1000 can detect a process deviation alarm or presence of excess noise due to vibration. Alert conditions can be checked, for example: limit switch status on computer controlled machinery; status of valve actuated switches; pressure limit switches; and operation of solid state switching devices with control machinery. The LM-1000 automatically reacts to deviations from set conditions by phoning, in sequence, up to four pre-programmed numbers until the message is received.
Circle No. 149 on Reader Service Card.

● A new range of safety coated glass carboys in sizes up to 12 gallons is available from Wheaton Safety Co. The 'second skin' glass carboys are coated with an impact resistant, high tear thermoplastic — transparent, translucent or opaque — providing impact protection to reduce breakage potential and increasing manual handling stability. In the event of container breakage, the coating safely contains dangerous chemicals, and radioactive and biohazardous materials long enough for proper disposal.
Circle No. 150 on Reader Service Card.

Filling jobs by special initiative

Richard Pearson*

The UK Government has boasted of success in encouraging the sunrise industries. Will the manpower be there to support growth?

JOB prospects in science and technology are in a period of rapid change. While new "discoveries" are one major driving force, their application to society's needs and a changing financial context are nowadays equally strong pressures affecting employment. Existing staff need new skills, some have to retrain, while new jobs and areas of employment are always emerging. In the United States, skill shortages in information technology (IT) and electronics have led to severe depletion in the education system of the teachers needed to educate and train future specialists.

In the United Kingdom, cutbacks in the university system in the past 3 years have led many universities to rely on natural wastage to reduce staff numbers. Not surprisingly, those with marketable skills in industry have often been the ones to leave, while the rash of early retirements and freezes on recruitment have led to unbalanced staffing structures and blocked career streams. In an effort to unblock the system, a "new blood" initiative was announced by the Department of Education and Science in late 1982 which enabled the universities to recruit more than 240 new, young, teaching and research staff. There were also funds for a further 70 staff in IT as part of a wider "IT initiative" to increase the supply of graduates coming onto the labour market. Initial evaluations of these two schemes are now available.

Altogether, more than 300 posts were created in the universities and 87 per cent of them had been filled by late 1983. The University Grants Committee (UGC) reported a wide variation in the number and quality of applications for individual posts. The number of applications was as high as 80 for some posts in medicine with many strong candidates, and as low as 4 in engineering. The highest quality was found in applicants in the biological sciences and mathematics, the lowest in engineering. Information technology had the lowest number of applicants per post. Not surprisingly, the posts in engineering and information technology were hardest to fill, with nearly one in five of the information technology posts, and one in three in engineering remaining vacant (see table).

The aim of attracting young staff was largely achieved; more than half the recruits were under 30, and more than 90 per cent under the recommended age limit of 35. Interestingly, one in ten of the information technology recruits was over 36, while three were under 25. In a year dedicated to "Women in Science and Engineering", none of the information technology posts and only one of the engineering posts was filled by a woman, although more were appointed in medicine (6) and the sciences (9).

New posts in UGC new blood and IT initiative

Subject	Posts in 1983	Applications per post	Posts unfilled at 30 Nov.1983
Medicine	43	19.7	5
Engineering	48	15.9	15
Biological sciences	25	32.1	3
Maths/ physical sciences	86	27.3	1
Agricultural/veterinary science	8	13.7	2
Arts/social studies	32	27.5	4
Information technology	70	10.9	13
Total	312	20.8	43

The IT initiative of 1982 also included additional funds for research and student places, as well as for a series of one year postgraduate and post-experience courses to increase the supply of people with IT skills. An initial evaluation of the first year of these courses, sponsored by the Science and Engineering Research Council (SERC), shows them to be making a rapid contribution to meeting recruitment needs despite their newness and a relatively low level of awareness by employers (*Manpower for Information Technology*, A. Gordon and R. Pearson, IMS). SERC is supporting two types of course, "conversion" courses aimed at graduates with no previous experience of information technology and "specialist" courses for information technology graduates.

Although many of the courses, run in both universities and polytechnics, were announced only a few months before they began in October 1982, there was a great deal of interest among first degree graduates — some course organizers received more than 300 applications for 20 or 30 places. Much of this enthusiasm was no doubt prompted by the poor job prospects for graduates without skills in information technology. Although there was a significant number of women applicants, they represented only one in eight students, possibly because many of the successful applicants had first degrees in engineering or physical science, often an essential entry requirement. The students themselves had mixed backgrounds; one in four were from polytechnics, and one in four had non-technical first degrees. Their ages ranged from 21 to 55, half were over 25 and officially classified as "mature" students.

The numbers graduating so far, fewer than 200, were relatively low when compared with the expected 1,000 graduates in 1984. In 1983 there was little awareness among employers of this new source of skilled information technology manpower. Most employers were seeking a broad based intake of first degree graduates and it was rare to find a significant discrete demand for MSc or diploma students in any of the information technology subjects. Some were, however, sought in communications and control engineering. In the case of postgraduates with first degrees in the arts, some employers doubted whether they could learn enough useful computing in one year to be worthwhile, although one major electronics company has recently organized his own three-month courses to turn arts graduates into successful real-time programmers. By the time they graduated, most students had found jobs that used their new skills, with banks and in education as well as in the information technology industry. Starting salaries ranged from £5,000 to more than £12,000 a year, with half in the £7,000-8,000 range, well above the average starting salary for new graduates, which in 1983 was just over £6,000.

The information technology initiative has been successful so far in creating a new supply of skills. Ironically it could be poor information that constrains this supply of information technology skills in the future, with some employers unaware of these new types of graduates, and some course organizers having little interest in ascertaining industry's needs. Women still remain a large untapped source of recruits for the courses and for industry. This always assumes of course that there will be enough teaching staff to run the courses. □

* Institute of Manpower Studies, Mantell Building, University of Sussex, Brighton BN1 9RF, UK.

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SAMUEL MILTON NABRIT MARCH OF DIMES PROFESSOR

Applications and/or nominations are invited for the Samuel Milton Nabrit March of Dimes Professorship. Candidates with substantial experience in techniques and concepts of molecular genetics and immunogenetics are preferred; although highly qualified investigators in other areas of molecular and cell biology will be considered. It is anticipated that the successful candidates will establish a biotechnology focused research program with emphasis on studies relevant to birth defects in collaboration with faculty colleagues of Atlanta University and Morehouse College. Salary will be commensurate with qualifications and experience.

Submit curriculum vitae, three letters of reference and a statement of current research interests by June 1, 1984 to: Dr Judith Rae Lumb, Department of Biology, Atlanta University, Atlanta, Georgia 30314.

The University is an Equal Opportunity/Affirmative Action Employer.

(NW495)A

KINGSTON POLYTECHNIC FACULTY OF SCIENCE RESEARCH ASSISTANT

The Department of Industrial, Organic and Polymer Chemistry invites applications from suitably qualified candidates to undertake an investigation into the synthesis and properties of thermally and hydrolytically stable crosslinked polymers. The work will include lamination and physical testing of resins prepared.

Candidates should have, or expect to obtain this year, a good honours degree in Chemistry. The successful candidate will be expected to register for a higher degree under the supervision of Dr W E Douglas. The post is for 3 years and involves some teaching duties. Salary within range £5,592 — £6,294 inclusive.

Details and application forms (to be returned by 31st May) from Academic Registry, Dept AO, quoting Ref SC, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. Tel: 01-549 1366 ext 518.

(1889)A

UNIVERSITY OF CALIFORNIA, IRVINE

FACULTY POSITION IN PHARMACOLOGY

Applications are invited for a tenured or tenure-track faculty position in Pharmacology. We are especially interested in candidates with a background in cardiovascular pharmacology, but qualified candidates in any area of molecular pharmacology will be considered. A Ph.D. in Pharmacology or related area, or an M.D., is required.

Level of appointment and salary will be commensurate with achievement and experience.

Curriculum vitae, references and a summary of research interests should be sent before June 30, 1984, to: Dr. Larry Stein, Chairman, Department of Pharmacology, College of Medicine, University of California, Irvine, California 92717.

The University of California is an Affirmative Action Employer.

(NW491)A

UNIVERSITY OF MANCHESTER MEDICAL SCHOOL

DEPARTMENT OF PHARMACOLOGY

POSTDOCTORAL RESEARCH ASSISTANT REPRODUCTIVE BIOLOGY

Applications are invited for a postdoctoral position funded by the Cancer Research Campaign initially for 1 year with a possible extension to 3 years. The research will investigate and develop treatments which protect the testes from drugs used for the treatment of cancer. Preference will be given to candidates with experience in histology and radioimmunoassay in reproductive biology. Salary on scale Research Fellow 1A £7630—8530 p.a. with superannuation. Starting date not later than October 1, 1984.

Applications and curriculum vitae (2 copies) with the names of two referees should be addressed to DR. I.D. MORRIS, Department of Pharmacology, Medical School, Oxford Road, Manchester M13 9PT, U.K.

(1888)A

Senior Pathologist

Pharmaceutical R&D

Loire Valley

Our client is one of the largest and most successful international pharmaceutical companies with research establishments in several European countries, including the UK and France. The company has its 100-strong European Safety Evaluation Laboratories in France, and it is here that this vacancy has arisen.

The successful candidate will be a senior member of a small team of pathologists supported by well-experienced technical staff. In addition to classical light and electron microscopy, the department is also making increasing use of histochemical and immunocytochemical techniques in the evaluation of animal pathology. Specialist experience in one or other of these fields would be desirable.

Candidates must have either a veterinary or medical qualification, and membership of the Royal College of Pathologists, American Board Certification in Pathology, or equivalent professional recognition. Some experience of laboratory animal pathology would be a distinct advantage, as would a specialist interest in haematology. Written and spoken fluency in English is essential, and the successful candidate, if not French, must be prepared to become proficient in French after a short period.

As one of the senior scientific staff of the Centre, there would be considerable interaction with research colleagues at other international company locations, and attendance at appropriate scientific congresses is encouraged.

A highly competitive starting salary will be negotiated according to qualifications and experience. Other benefits include pension and life assurance schemes, up to 30 days' annual leave, and assistance with international relocation expenses.

Please write in confidence for an application form, or phone if you would like to discuss the appointment further before applying:

Talentmark
Leaders in Health Care Recruitment

James A Edwards, Talentmark Limited, King House, 5-11 Westbourne Grove, London W2 4UA. Tel: 01-229 2266.

(1926)A

Experienced Pharmacologist/ Physiologist

Internationally respected as a leading name in the field of ethical pharmaceuticals, Smith Kline and French Research operates an intensive and innovative research programme. Due to an internal transfer we now have a vacancy for a Pharmacologist/Physiologist within one of our Pharmacology departments.

The successful candidate will be responsible for carrying out and reporting experiments designed to study the pharmacological and electrophysiological actions of drugs and novel compounds on the heart.

We are looking for a PhD, or a candidate with a good honours degree in Pharmacology or Physiology. Of primary importance however is proven experience in cardiac electrophysiological techniques.

We pay competitive salaries and provide a full range of benefits. Please write for an application form or send a detailed c.v. outlining your qualifications and experience, quoting reference number N/965/SB to the Personnel Officer.

SK&F

SMITH KLINE &FRENCH RESEARCH LIMITED
The Frythe, Welwyn, Herts AL6 9AR.

(1952)A

LUND UNIVERSITY, SWEDEN
announces a permanent professorship in THEORETICAL ECOLOGY at the mathemactic-natural sciences faculty

The scientific area includes ecological processes and interactions of general character on the population and ecosystem levels as well as the environmental and evolutionary background of the ecosystem's variation and development. Important parts of the area are formulation of models and hypotheses in correspondence with empirical research.

The appointee is expected to make contribution towards stimulation of the present research programmes and to develop new lines of investigation, in close cooperation with his/her fellow professors in the department. Teaching responsibilities and supervising postgraduate students.

Reference number 5440.

Principles of promotion for a professorship are mentioned in chapter 19, paragraph 13 of the University edict.

The application letter should be addressed to the Swedish Government (Ref nr) but sent to the Registrar, Lund University, PO Box 1703, S-221 01 Lund, Sweden.

Deadline for receipt of materials: May 23. Starting salary: 146,232 – 165,780 Sw. Cr. per annum (before taxes).

The applicant should include the following items in the application:
1. short written summary of his/her scientific and pedagogic activity (1 copy); 2. a witnessed (two names) list of merits and a list of scientific publications (1 copy); 3. a copy of transcripts or testemonials and other papers of recommendation (1 copy); 4. 4 copies of each scientific work – numbered as in the list above (2), which should be separated into 4 packages for review by the selection committee (at the latest within 3 weeks after the deadline).

(W952)A

THE UNIVERSITY OF NEW ENGLAND CHAIR APPOINTMENTS

The University of New England invites applications for the following tenured Chairs:

PHYSIOLOGY – Vacant following the retirement of Professor J. V. Evans. The Department offers courses in Physiology in the Faculties of Science and Rural Science and in Human Bioscience in the Faculty of Arts.

ZOOLOGY – Vacant following the retirement of Professor A. F. O'Farrell. The Department offers a range of courses with emphasis on ecologically-oriented subjects.

Each of the Departments teaches both internal and external students (the latter being required to attend residential schools). Both Departments have active research programmes and supervise postgraduate student research.

In each case the appointee will become Head of Department for an initial period of five years.

The professorial salary is A$48,997 per annum. Academic salaries are at present under review.

Full particulars can be obtained from the Staff Officer, University of New England, Armidale, New South Wales, 2351, Australia, Telephone ISD 61 67 73 2100, with whom applications, which should include the names and addresses of three referees, close on 21st June, 1984.

Informal enquiries may be directed to the Chairman of the Academic Board, Professor I. R. Falconer, Telephone ISD 61 67 73 2938.

(W941)A

Research Opportunity in Marine Physics

The Institute of Oceanographic Sciences has a vacancy at Scientific Officer level with its Marine Physics Group. The work is concerned with ocean circulation and will entail extensive use of computers for the calibration, analysis and presentation of data from recording instruments deployed in the deep ocean.

Candidates should be numerate, preferably with a mathematics and/or physics background. Previous experience with computers is not essential. Applicants should have a degree, HNC/HND or degree standard qualification in an appropriate scientific or engineering subject.

Although there is only one vacancy it may, if suitable candidates are available, be split to provide two part-time posts.

Starting salary will depend on qualifications and experience, but will be in the range of £5,682 pa – £7,765 pa (full-time rates quoted).

Application forms and further details are available from: The Secretary, Institute of Oceanographic Sciences, Brook Road, Wormley, Godalming, Surrey GU8 5UB. Telephone: Wormley (042879) 4141 ext 272. Please quote Reference 84/004/106. Closing date for applications: 18 May 1984.

Natural Environment Research Council

(1881)A

Opportunities in Pharmaceutical Development

Pathology Division — *Ware, Herts*

Glaxo Group Research is Britain's leading pharmaceutical research organisation, with a distinguished reputation for developing safe and effective medicines. Due to an expanding workload, two positions have arisen in our Pathology Division.

Cytogeneticist

We require a good honours graduate to join our team of Genetic Toxicologists. You will be involved in cytogenetic analysis including metaphase chromosome studies of mammalian cells. You will be expected to assist in the preparation of the specimens for analysis and in the assessment of results. The laboratories are purpose built to the highest standard and equipped with the latest computer technology.

Applications are invited from graduate cytogeneticists with some experience of metaphase analysis.

Reproductive Toxicologist

We require a graduate to be a study supervisor in our Toxicology Department working in the team assessing the safety of new drugs when used in pregnancy.

You will be involved in the implementation of reproductive toxicology studies and ensuring the correct execution of the studies according to GLP requirements.

You will be expected to be fully aware of the status of each study, as it proceeds, by collating and correlating data generated by the department's dedicated computer system.

Candidates should possess a relevant good honours degree together with a sound knowledge of animal anatomy. Experience gained in this field of work would be a distinct advantage.

We offer a competitive salary in the range £6,175-£10,770. Your starting salary will reflect your experience. Other benefits of a highly successful company include participation in Glaxo Group profitability, non-contributory pension scheme, an active sports and social club and, where appropriate, assistance with relocation to this pleasant area of Hertfordshire.

Please write or telephone for an application form, stating which vacancy interests you to: Mr. D. Latter, Personnel Officer, Glaxo Group Research Ltd., Ware, Herts. SG12 0DJ. Tel: Ware (0920) 3993.

Glaxo *Group Research Ltd.*

(1954)A

Rhodes University, Grahamstown, South Africa

Applications are invited from suitably qualified candidates, irrespective of race, colour or creed, for the following post, from 1 January 1985 or as soon as possible thereafter:

Lecturer/Junior Lecturer in Entomology

in the Department of Zoology and Entomology

An interest in biological control, insect ecology, or plan/insect relationships may be a recommendation.

The salary scales are:

Lecturer : R12657 × 780 - 16557 × 936 - 22173 per annum

Junior Lecturer: R9075 × 621 - 10317 × 780 - 14217 per annum

In addition to the above salaries, an allowance of approximately 12% is payable. The initial salary will be determined according to qualifications and experience. Fringe benefits include generous leave privileges, financial assistance towards the University education of children at Rhodes University, and a service bonus subject to regulations. The successful applicant will become a member of the University's pension and medical aid schemes and qualify for a housing subsidy subject to regulations.

Application forms and further particulars may be obtained from The South African Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE to whom complete applications should be sent by not later than 31 May 1984. (W964)A

Southampton THE UNIVERSITY

SYNTHETIC ORGANIC CHEMIST

Applications are invited for a position of postdoctoral Research Scientist in the Wolfson Unit of Chemical Entomology from 1st October, 1984. The major role would be synthesis of insect behaviour modifying chemicals for use in research programmes leading to new and improved methods of insect control. The Unit is a self-funding consultancy comprising both biologists and chemists working in close cooperation with government and industrial research establishments towards the development of novel pest control strategies. Salary, which will depend upon age and qualifications, will be a minimum of £7,190 per annum (under review). Applications, together with a curriculum vitae and the names and addresses of two referees, should be sent as soon as possible to Professor R. Baker, Department of Chemistry, The University, Southampton SO9 5NH. (1922)A

Molecular Biologist SC/L2/84/1

ILRAD wishes to recruit a senior scientist to lead its molecular biology programme in theileriosis, trypanosomiasis and bovine lymphocyte antigens. The research group includes 3-4 post-doctoral fellows with senior technical support. ILRAD has a mandate to devise improved control measures for animal diseases and the molecular biology programme plays an important role in the multidisciplinary effort. ILRAD is fully funded by an international group of donor agencies and is equipped to the highest standards.

Current research topics include genetic mechanisms controlling antigenic variation in salivarian trypanosomes, identification of gene sequences which identify species, sub-species and serodemes of trypanosomes, identification of genes coding for protective antigens of trypanosoma and theileria parasites, and analysis of genetic changes in host and parasite associated with lymphocyte proliferation induced by parasitic infection.

Candidates should have a good research record in molecular biology, a minimum of 7 years research experience since obtaining PhD, and proven ability to head a research group.

This position is an international staff position and salary will be paid in US dollars. Initial salary level will be determined by qualification and experience. Moving, housing, commutation and education allowances are paid, and there are medical and retirement benefits. Applications should include a current curriculum vitae and the names and addresses of three referees. Applications, citing the reference number should be sent by 30th June 1984 to:

Chief Personnel Officer,
I.L.R.A.D.
PO Box 30709
NAIROBI
Kenya

(W954)A

UNIVERSITY OF WAIKATO
New Zealand
APPOINTMENTS IN PHYSICS

Applications are invited for a junior lectureship and a lectureship within the Department of Physics. The appointment to the junior lectureship will be for not more than four years.

The successful applicant for the junior lectureship should have either an MSc in physics or a BE in electrical or electronic engineering. The appointee should have basic interests in micro-processors and semi-conductor devices.

The appointee to the lectureship should have a PhD in physics in an electronics area, and would be responsible for the teaching of electronics and associated supervision of MSc students.

The present research interests of the Department of Physics include: Plasma Physics, Theoretical Physics, Solid State Physics, Biophysics and Electronic Instrumentation.

The appointments will be made at an appropriate step on the scale for junior lecturers, which is NZ$16,123 — NZ$18,732 per annum and for lecturers NZ$21,660 — NZ$25,684 per annum.

All enquiries, including details of the prescribed method of application and conditions of appointment, should be addressed to the Registrar, University of Waikato, Private Bag, Hamilton, New Zealand, or the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close with the Registrar on 31 May 1984. (W960)A

MASSEY UNIVERSITY
Palmerston North,
New Zealand
FERTILIZER AND LIME RESEARCH CENTRE

Applications are invited for the position of

SENIOR RESEARCH OFFICER

in the Fertilizer and Lime Research Centre which is based in the Department of Soil Science. The Centre was established in January 1983 and undertakes research on several aspects of fertilizers, including the development and evaluation of new and improved fertilizer materials and the efficiency of fertilizer use.

The successful applicant will be required to undertake and coordinate research in areas relevant to the interests of the Centre. Some assistance with research administration will be required.

Applicants must have appropriate post-graduate qualifications and at least five years experience in soil fertility-related research. A good publication record and some experience and an interest in research coordination and administration would be desirable.

An appointment at a salary of up to NZ$30,127 per annum is possible depending on qualifications and experience and will be for 3 years in the first instance.

Further details of the position and of the University, together with conditions of appointment, may be obtained from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University with whom applications close on 30 May 1984. (W959)A

vrije universiteit
amsterdam

The **Subfaculty of Chemistry, Department of Pharmacochemistry** has a vacancy for a

full professor in (molecular) toxicology (male/female)

The department takes care of teaching in pharmacochemistry and is involved in the program for "environmental chemistry". The research program mainly concerns neutrotransmitters. For both the teaching and the research program in pharmacochemistry the subjects are treated in an integrated way, in which chemicals (synthesis, physical-chemistry) and biological (pharmacology, pharmacokinetics, metabolism, toxicology) aspects are involved: for the biological investigations approaches on the molecular level are followed. Additionally the study of quantitative structure-activity relationships is part of the program.

The applicant should have a broad experience in the field of pharmacology, especially of toxicology. He should be able to contribute to the teaching program of the department and to stimulate the development of the research program on molecular toxicology.
He should be willing and able to take part in the administrative tasks of the department and the subfaculty.

The salary will be between Hfl.76.100,- and Hfl. 108.100,-- per annum (excl. 7,5% holiday allowance). Candidates should be prepared to identify with the Christian character of the Free University.

Those requiring further particulars and those wanting to draw attention to suitable candidates are asked to contact the chairman of the nomination committee, prof.dr. H. Timmerman (Department of Pharmacochemistry of the Free University, De Boelelaan 1083, 1081 HV Amsterdam, telephone 020-548 57 26).

Written applications, including a curriculum vitae and a list of publications should reach the Free University within a month after the date of appearance of this advertisement. (Personnel Department, P.O. Box 7161, 1007 MC Amsterdam, The Netherlands). Please quote reference number 610-0584.

(W943)A

VU

UNIVERSITY OF LIVERPOOL

'NEW BLOOD' LECTURESHIP IN PHARMACOLOGY AND THERAPEUTICS

Applications are invited for the post of Lecturer in the Department of Pharmacology and Therapeutics, established under the UGC 'New Blood' initiative and tenable from 1st October 1984. Candidates should normally be aged 35 or under on appointment. Persons holding permanent university appointments in the UK, of whatever kind, are ineligible for appointment to this post. 'New Blood' appointments will be primarily for research.

Candidates should have a special interest in immunopharmacology, and would work closely with established biochemical and clinical pharmacology groups in the Department on problems of drug toxicity.

The initial Salary will be at an appropriate point within a specified range on the Lecturer scale (£7,190 — £14,125 per annum).

Applications, together with the names of three referees should be received not later than 31 May 1984, by the Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/296/N.

(1875)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF MEDICINE

POSTDOCTORAL RESEARCH ASSISTANT

required for up to 3 years to work with Dr Gregory Gregoriadis on a project supported by the MRC on the use of liposomes as immunological adjuvants in vaccines. Strong background in immunology essential/ experience of liposome technology an advantage.

Salary on Research Range 1A. Application by full curriculum vitae, quoting the names and addresses of two referees. Reference RA/GG.

TECHNICIAN

also required to assist on project. Candidates must hold relevant technical/academic qualifications and have at least two years working experience. Familiarity with immunological techniques an advantage.

Salary on scale £5,491 — £7,098 plus £1,042 London Allowance. Application forms available. Reference T/MU.

Job decriptions for both posts available from the School Office, RFHSM, Rowland Hill Street, London NW3 2PF to which application as above should be submitted by 18 May 1984. Please quote appropriate reference.

(1894)A

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

Scientist

Crop Research Division
Lincoln
New Zealand

A geneticist/plant breeder is required in an expanding plant genetics group supporting the established plant breeding programme at Crop Research Division, Lincoln.

Experience and knowledge of plant reproductive biology and genetics is required. Particular expertise in interspecific and/or intergeneric hybridisation, embryo culture, tissue culture and incompatibility in crop plants, especially cereals would be desirable. An applicant with an appreciation of the utility of these techniques to plant breeding programmes could take a leadership role.

An appropriate degree plus substantial research experience essential.

Salary up to NZ$35,731 would be determined according to qualifications and experience.

Further information, application forms, etc, may be obtained from the Migration Branch, New Zealand High Commission, New Zealand House, Haymarket, London SW1Y 4TQ. Applicants should quote reference number Imm 2/ 335/ 54 and forward applications accompanied by a résumé to:

The Migration Branch,
New Zealand High Commission,
New Zealand House, Haymarket,
London SW1Y 4TQ.

Closing date for applications June 15th, 1984.

(W949)A

Experienced Synthetic Organic Chemist

As a leader in the international pharmaceutical field, Smith Kline & French Research Limited has maintained an impressive record of growth, based on a commitment to research and development. Within our Synthetic and Isotope Chemistry Department, we now have an opportunity for an experienced Chemist to fill a senior position which has become vacant through internal transfer.

Applicants should possess a PhD degree in Chemistry with a broad knowledge, including practical experience, of many fields of synthetic organic chemistry. They must also have had a minimum of three years post doctoral experience in industry or academia with a proven track record of creativity and innovation to their credit.

Reporting to the Head of Department the successful candidate will be responsible for leading a team of graduate organic chemists engaged in all aspects of devising new synthetic procedures to medicinally active compounds.

We pay competitive salaries and offer a full range of benefits, which include free BUPA, free life assurance and flexible working hours.

Please forward a detailed CV outlining qualifications and experience, or contact the Personnel Officer for an application form, quoting reference number N/964/SB

SK&F

SMITH KLINE &FRENCH RESEARCH LIMITED
The Frythe, Welwyn, Herts AL6 9AR. (1887)A

Harpenden Laboratory, Herts
Ministry of Agriculture, Fisheries and Food

Mass Spectroscopist (Organic)

...to work on problems associated with pest control and environmental pollution by identifying pesticide residues and other low level contaminants in plant, animal and other substrates using gc-ms, lc-ms, EI/CI, FAB, positive/negative ion-ms and other relevant techniques.

Candidates should have a good degree (or equivalent) in chemistry, biochemistry or other relevant subject. Experience in the techniques used and an interest or experience in computer techniques and/or electronics will be an advantage.

Appointment as Scientific Officer £5680 – £7765 with starting salary according to qualifications and experience.

For further details and an application form (to be returned by 24 May 1984) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours).

Please quote ref: SB/29/AG.

The Civil Service is an equal opportunity employer.

(1951)A **Scientific Civil Service**

ASSISTANT/ ASSOCIATE PROFESSOR
(tenure track)
DEPARTMENT OF MEDICINE
STANFORD

The Division of Oncology will be appointing a laboratory based medical oncologist at Stanford. We are searching for an individual with independent research capability and a record of productivity, preferably in the areas of molecular biology as it relates to viral oncology, immunology, genetics or chemotherapy of neoplasia. In addition, this individual will be expected to participate in teaching programs and patient care in medical oncology.

Send résumé, including bibliography and summary of research goals, to: Dr Ronald Levy, Chairman, Search Committee, Division of Oncology, Stanford University School of Medicine, Room SO25, Stanford, California 94305. Stanford is an Equal Opportunity Employer and welcomes nominations of women and minority group members and applications from them. (NW504)A

Southampton THE UNIVERSITY

NEW BLOOD LECTURESHIP ECONOMIC GEOLOGY

Applications are invited for the post of Lecturer in Economic Geology with particular emphasis on the geochemistry of fluid-rock interactions.

Salary scale: £7,190 × £450 (15) – £14,125 per annum (under review). The initial salary will depend on qualifications and experience.

Further particulars may be obtained from Mr D. A. S. Copland, The University, Southampton SO9 5NH to whom applications (7 copies from United Kingdom applicants) should be sent not later than 25 May 1984, quoting reference no: 2039/N. (1890)A

POST DOCTORAL POSITIONS

Two positions available immediately for PhD graduates interested in cellular and molecular aspects of early mammalian development, introduction of exogenous genetic material into mouse embryos and/or the relationship between embryonic and tumour cells. Bankground in molecular biology, cell culture or developmental biology required. Salary $20,000 per annum.

Positions tenable at Brock University till July, 1985, and then at the new Mount Sinai Research Institute, associated with the University of Toronto. Apply to Dr J Rossant, Dept of Biological Sciences, Brock University, St Catharines, Ontario, Canada L2S 3A1. (NW502)A

The Queen's University of Belfast
NEW LECTURESHIPS

Applications are invited from persons under the age of 35 years for the following new lectureships tenable from 1 October 1984.

INORGANIC CHEMISTRY

Applications are particularly welcome from candidates with interests in the early transition elements, high oxidation state transition metal chemistry, or organometallic chemistry. However, well qualified candidates with other fields of interest are encouraged to apply. Closing date: 15 June 1984.

THEORETICAL ATOMIC PHYSICS OR ASTROPHYSICS

This post is in the Department of Applied Mathematics and Theoretical Physics. Preference will be given to candidates having experience and a strong research interest in theoretical atomic and molecular physics and/or astrophysical plasmas. Closing date: 31 May 1984.

Salary scale for both posts is £7,191 – £14,127 per annum with contributory pension rights under USS, initial placing on scale depending on age, qualifications and experience.

Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast BT7 1NN, Northern Ireland. Closing date: 15 June 1984. (Please quote ref. 84/N). (1916)A

STANFORD UNIVERSITY
seeks candidates for a newly endowed Chair:

The Henry S. Kaplan Professorship in Cancer Biology at the School of Medicine

We wish to identify a distinguished senior scientist who has demonstrated outstanding scholarship in areas related to cancer biology. The appointee would be expected to assume the Directorship of the Cancer Biology Research Laboratory and its associated research training activities.

Please send nominations and information relevant to the applicant's qualifications to the Staff Assistant for the Search Committee, Ms Marian Knox, Dean's Office, Room M-119, Stanford University Medical Center, Stanford, CA 94305. Stanford University is an Equal Opportunity Employer and welcomes nominations of women and minority group members and applications from them. (NW505)A

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
DIVISION OF IMMUNOLOGY

Applications are invited for a postdoctoral position in the Division of Immunology to work with Dr B. A. Askonas. The appointment will be for one year, from 1st August 1984, to work on aspects of cell mediated immunity to influenza virus in a mouse model system. Experience in t-cell cloning and tissue culture of lymphoid cells is essential.

The salary will be in the range £8,530 – £10,250 per annum plus £1,186 London allowance.

Applications should include a curriculum vitae and the names of two referees and should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA before 24th May 1984 quoting ref: IMM/9/P. (1904)A

Southampton THE UNIVERSITY

DEPARTMENT OF MICROBIOLOGY
Applications are invited for a NEW BLOOD LECTURESHIP IN MOLELCULAR GENETICS

Candidates should have a PhD and post-graduate experience in a relevant field of molecular biology. Salary scale: £7,190 × £450 (15) – £14,125 per annum (under review). The initial salary will depend on qualifications and experience. Further particulars may be obtained from D. A. S. Copland, The University, Southampton, SO9 5NH to whom applications (7 copies from United Kingdom applicants) should be sent not later than 30 May, 1984, quoting reference number 2041. (1896)A

UNIVERSITY OF CAMBRIDGE
'NEW BLOOD' LECTURERSHIP IN BIOTECHNOLOGY

Applications are invited for a University Lectureship or University Assistant Lectureship in Biotechnology to commence on 1 October 1984. Candidates should be graduates in chemistry, biochemistry, biophysics or a relevant subject with previous experience in electrochemistry. The person appointed will be expected to contribute to the development of teaching strategies and to complement the active research programme in biosensors/bioelectronics being pursued in the newly established Biotechnology Centre. Industrial experience will be welcome.

Further information about the duties and conditions of appointment may be obtained from the Director of Biotechnology, The Biotechnology Centre, Downing Street, Cambridge CB2 3EF, to whom applications, including a Curriculum Vitae, list of publications, and the names of three referees, should be sent so as to reach him not later than 18 May 1984. (1917)A

THE UNIVERSITY OF LEEDS

DEPARTMENT OF PHYSIOLOGY

LECTURER

Applications are invited for the above post available from 1 October 1984.

Qualifications in the field of neurophysiology with research interests related to visceral sensory mechanisms are required.

Salary on the academic scale for Lecturers (£7,190 – £14,125) (under review) according age, qualifications and experience.

Informal enquiries may be made to Professor B R Jewell (tel Leeds 431751 ext 7267).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT quoting reference no. 104/11/D. Closing date for applications 21st May 1984. (1910)A

The Queen's University of Belfast
NEW LECTURESHIP IN MOLECULAR BIOLOGY (NON-CLINICAL)
Department of Medical Genetics

Applications preferably from candidates under 35 years of age are invited for a lectureship (non-clinical) in the Department of Medical Genetics tenable from 1 October 1984 or such other date as may be arranged. Applicants should preferably possess a PhD degree or equivalent, and must have experience in recombinant DNA techniques. The lecturer will be expected to help the department expand its interest in the application of molecular biological techniques to human disease and clinical genetics. Initial placing, which will depend on age, qualifications and experience, will be made on the scale for lecturers: £7,191 – £14,127 with contributory pension rights under USS.

Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast BT7 1NN, Northern Ireland. Closing date: 31 May 1984. (Please quote Ref. 84/N). (1914)A

CSIRO

Research Fellow

$A24,344 – $A35,806
Division of Atmospheric Research
Aspendale VIC
Australia

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Atmospheric Research is a member of the Institute of Physical Sciences.

Field: Atmospheric Clouds and Radiation.

General: The Division of Atmospheric Research maintains broadly-based research programs in dynamical meteorology, boundary layer physics, climatology, radiation, cloud physics and atmospheric chemistry. Total staff numbers approximate 130.

The Division's Clouds and Radiation Group is concerned with the meteorological and climatic consequences of the interaction of atmospheric radiation with clouds, aerosols and trace gases, the physics of cloud evolution, including the investigation of natural rainforming processes, the remote sensing of atmospheric and surface parameters by lidar, satellite and airborne radiometric techniques. Recently the group has acquired a comprehensive system for real-time acquisition and interactive handling of high resolution meteorological satellite data (CSIDA).

Duties: As a member of the Clouds and Radiation Group, the appointee wil have considerable freedom to develop and undertake research within the general field. There is also a requirement to strengthen the theoretical background to the Group's present activities and, specifically, to collaborate in research concerned with interpretation of satellite radiances and with investigation of those radiative processes that are affected by the behaviour of clouds, aerosols and by changes in atmospheric trace gases.

Qualifications: A PhD degree or equivalent qualifications with emphasis on theoretical physics and some training in applied mathematics plus proven research ability. Experience in general meteorology and, in particular, the theory of radiative transfer would be an advantage.

Tenure: Indefinite with Australian Government superannuation benefits available.

Applications: Stating full personal and professional details, the names of at least two referees and quoting reference No A1824, should be directed to:

The Chief,
CSIRO Division of Atmospheric Research
Private Bag No 1
Mordialloc VIC 3195
Australia.

By June 8, 1984.

CSIRO is an equal opportunity employer. (W942)A

Athrofa Gogledd Ddwyrain Cymru
The North East Wales Institute
of higher education

CONNAH'S QUAY, DEESIDE, CLWYD

BIOTECHNOLOGY RESEARCH POST

(up to £13,443 p.a.)

The Institute's research programme already includes a substantial amount of work on immunoassays and on monoclonal antibodies. It now wishes to increase its involvement in biotechnology especially in view of its support to Newtech, Clwyd's new Industrial Innovation Centre.

The person appointed will join the Academic Research Staff, will be required to undertake and initiate applied research, and to advise local industry. Particular areas of interest are in cloning, immunoassays or cell biology. Applicants should be professional researchers of high academic ability, and should be willing to apply their work to industrial objectives. Salary is between £8,937 to £13,443 pa depending on qualifications and experience. For further details and application forms write to:

The Registrar, The North East Wales Institute, Connah's Quay, Deeside, Clwyd. Tel: Deeside 817531 ext 254. Closing date for receipt of applications: 15th May, 1984. (1878)A

Statistician

c.£13,000 inc. (under review)

Glaxo Pharmaceuticals, one of the fastest growing companies in the UK, markets a highly successful range of leading pharmaceutical products.

We are seeking to appoint a statistician to advise on the statistical aspects of Clinical Trials protocols.

This work will involve an input into the design of protocols, record forms and computer entry screens to ensure that the data can be handled efficiently.

You will be required to analyse clinical data using appropriate methods and computer techniques.

In order to fulfil these responsibilities you will need to liaise with Management Services Division as well as members of Clinical Research Division and other Medical staff.

A strong positive personality is required and you should have considerable experience of the statistical aspects of clinical trial design together with the ability to gain the confidence of Senior Medical Personnel and to co-operate with them.

Attractive conditions of employment include Company bonus scheme, non-contributory pension scheme and other excellent benefits.

Please call for an application form to Mr. M. E. Bates, Senior Personnel Officer,

Glaxo Pharmaceuticals LIMITED
GREENFORD ROAD, GREENFORD, MIDDX. TEL: 01-422 3434, *quoting ref MEB/733.* (1953)A

MERCK SHARP
& DOHME-CHIBRET

SUBSIDIARY OF MERCK AND CO.

continues to develop its new Research Center
Located in the heart of France at
RIOM (PUY DE DOME)
and seeks for its
SAFETY ASSESSMENT DEPARTEMENT

1 PATHOLOGIST
2 TOXICOLOGISTS

Applicants with a DVM, MD, PhD or equivalent and adequate postdoctoral training should have 5 years experience and relevant publications.

A professional environment is offered in staff equipment and facilities. Candidates should have French as their conversational language and a good working knowledge of English.

MSD
MERCK SHARP & DOHME CHIBRET

Hand-written applications with a detailed curriculum vitae and expectations should be addressed to : Direction du Personnel
Route de Marsat
63203 RIOM Cedex - FRANCE.

(W948)A

UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST. THOMAS'S HOSPITALS

OCCUPATIONAL HEALTH RESEARCH UNIT
DEPARTMENT OF COMMUNITY MEDICINE

RESEARCH ASSISTANT

required for one year to join a team at Guy's investigating the occupational aspects of low back pain, funded by the Arthritis and Rheumatism Council.

Applicants should have a degree in a human science subject with a basic knowledge of biomedical engineering and computing. Research experience and an interest in ergonomics is desirable but not essential. If further information is required regarding this post please contact E Otun on 01-407 7600 Ext 3108.

Salary in range £6,310 — £8,530 according to qualifications and experience, plus £1,186 London Allowance and superannuation in USS.

Apply in writing with full curriculum vitae, to the Secretary, United Medical and Dental Schools, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref CM1. Closing date for applications 17th May 1984. (1940)A

THE SCHOOL OF PHARMACY
UNIVERSITY OF LONDON

LECTURESHIP IN PHARMACEUTICAL CHEMISTRY (SPECIALIST IN BIOCHEMISTRY OR PHARMACEUTICAL BIOCHEMISTRY)

Applications are invited from per sons with training and interests i biochemistry and its applications i the pharmaceutical, biological an health-related sciences. Examples o suitable research areas are mem branes and receptors, metabolism enzymes, gene cloning, macromole cule-drug interaction, oncology an toxicology. The successful candidat will participate in the teaching o BPharm and BSc (Toxicology Pharmacology) degrees.

Salary in range £7190 — £14125 London Allowance (£1186) p.a.

Further details from Profess Gibbons, Department of Pharm ceutical Chemistry (address below).

Applications (6 copies) with Cu riculum Vitae and names of referees, quoting ref JM/N, to: Pe sonnel Officer, The School of Pha macy, University of London, 29/ Brunswick Square, London WC1 1AX (Tel: 01-837 7651). (1885)A

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF CHEMICAL ENGINEERING AND CHEMICAL TECHNOLOGY

POSTDOCTORAL RESEARCH ASSISTANT AND RESEARCH ASSISTANT IN BIOTECHNOLOGY

Applications are invited for two posts to assist in a growing programme of research into the production of fuels and chemicals by fermentation. The work is supported by The Royal Society.

Candidates for the postdoctoral post may be qualified in biochemical engineering or applied microbiology and should have experience and interests relevant to the work of the group which presently includes anaerobic digestion, immobilised cell systems and coupled fermentation and novel separation processes. Initial salary according to age and experience in range £7190-£11615 plus £1186 London Allowance.

Candidates for the post of research assistant should be graduates, preferably in chemical engineering, and have some experience of work in a biochemical or microbiological laboratory. The successful candidate will assist in work on mixing, modelling and evaluation of anaerobic fermentations and will also be required to provide general technical assistance in the biotechnology laboratory. Salary in range £6310-£8530 plus London Allowance according to age and experience.

Applications, naming two referees, should be addressed to Dr D L Pyle, Department of Chemical Engineering and Chemical Technology, Imperial College, London SW7 2BY, to arrive as soon as possible. Informal enquiries also welcome (01-589 5111 ext 1909).

(1949)A

SOUTH MANCHESTER HEALTH AUTHORITY CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE PATERSON LABORATORIES

A vacancy exists at postdoctoral level within a group devoted to the study of the epithelial cells of a variety of tissue. The interests of the group centre on the cellular organisation of proliferation in a wide range of surface epithelia (skin, oral mucosa and gastrointestinal tract), the mechanisms involved in the control of cell proliferation and differentiation and the malfunction of these controls that result in cancer and other proliferative disorders. We wish to bring new scientific interests into the group to add to the wide-ranging approaches that are currently used, and would be particularly interested in someone who could bring experience of monoclonal antibodies or molecular biology techniques or who would be interested in learning these techniques while working in the group. The successful candidate would be able to develop his own area of study within the overall framework and under the general guidance of Dr C S Potten, the group leader. Potential projects could include the identification of early differentiation events in epithelial cell lineages; characterisation of stem cells and clonogenic cells in epithelial tissues and their relationship to carcinogen target cells.

The work is supported by grants from the Cancer Research Campaign and the position would be tenable for three years with the possibility of renewal.

Applications should be made to Dr C S Potten, The Paterson Laboratories, Christie Hospital and Holt Radium Institute, Wilmslow Road, Manchester M20 9BX and include a curriculum vitae, the names of two academic referees and outline of interests in connection with the work outlined above. (1931)A

UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST. THOMAS'S HOSPITALS

POST-DOCTORAL RESEARCH ASSISTANT

required for one year to work on cell-mediated immune mechanisms in rheumatoid arthritis. Experience in cloning and hybridoma techniques would be desirable but not essential. This post is funded to 30th June 1985. Salary on the scale of £7,190-£8,530 plus £1,186 London Allowance and superannuation depending on age and experience. For further details 'phone Professor G S Panayi (01-407 7600 Ext 3269). Applications to the Secretary, United Medical and Dental Schools, Guy's Hospital Medical School, London Bridge, London SE1 9RT, quoting Ref DM6.

(1945)A

KINGSTON POLYTECHNIC

SCHOOL OF CHEMICAL AND PHYSICAL SCIENCES

SENIOR TECHNICIAN

A graduate in biochemistry or related discipline with bacteriological experience is required to be responsible for the operation of a biochemistry and bacteriology laboratory concerned with undergraduate, postgraduate and research work. Analytical biochemistry forms a major part of this work and experience of a wide range of appropriate instrumental techniques is required as well as routine maintenance of the laboratory. Salary scale 4/5 £6,891 — £8,523 inclusive.

Details and application forms (to be returned by 29th May) from Assistant Registrar (Personnel), Kingston Polytechnic, Penrhyn Road, Kingston upon Thames. Tel: 01-549 1366. (1927)A

AUSTRALIA

ROYAL MELBOURNE INSTITUTE OF TECHNOLOGY LIMITED

SENIOR LECTURER (INSTRUMENT SCIENCE)

DEPARTMENT OF APPLIED PHYSICS

REF. NO. 121/06/AN

Applicants are called for the above position. The successful applicant will be responsible for providing leadership to the Instrument Science Group, undertake a variety of teaching duties, and be expected to participate in the overall research and development program of the department.

It is expected that the successful applicant will hold a Ph.D., have extensive experience in tertiary teaching, and have an established research and/or development record associated with instrument science and microprocessor application.

Salary within the range $A31,390 — $A36,585 p.a. (under review).

LECTURER (OPTICS)

DEPARTMENT OF APPLIED PHYSICS

REF. NO. 121/09/AN

The Department is seeking an energetic applicant who will join the Optics group in the above position. The successful applicant will be expected to participate in undergraduate and postgraduate teaching programs and contribute to the research activities of the Optics group within the overall departmental research and development program.

It is anticipated that the successful applicant will hold a Ph.D. in an appropriate field and have demonstrated a research capability and commitment to the field of optics. Tertiary teaching experience would be an advantage.

Salary within the range $A23,394-$A30,734 p.a. (under review).

Position descriptions should be obtained from Staff Branch — (03) 341 2337. Applications quoting appropriate Ref. No. to the Staff Officer, RMIT, GPO Box 2476V, Melbourne, 3001, Victoria, Australia by 27 July 1984.

46099

(W940)A

BIO-COM

BIO-COM (U.K.) LIMITED

Bio-Com seeks for international research associates young independent and ambitious scientist with strong friendly personality, interested in both fundamental and development research. Training: immunology, genetic engineering, peptide-nucleic acid characterization. Initially based at Paris Institution, hence, conversational French essential. Willing to travel/relocate UK/USA. Starting salary £9,000 — £12,000.

Send cv, photograph and manuscript letter to the Director of Research, Bio-Com (UK) Ltd, 34 Floral Street, London WC2E 9DJ.

(W945)A

WELLCOME BIOTECHNOLOGY LIMITED, FMD DIVISION, PIRBRIGHT

SENIOR LABORATORY TECHNICIAN

A vacancy has arisen for a Senior Laboratory Technician to join our Research Team in the Foot and Mouth Disease Virus Section at Pirbright. This Section is part of our Department of Virology R&D at Beckenham and there is considerable interaction with other Biological Research Departments as well as collaboration with external organisations.

Applicants should be highly motivated, educated to degree or HTEC level and have the practical experience to provide full technical expertise required to general full length DNA clones of Foot and Mouth Disease Virus RNA. To obtain infectious virus from such clones and to perform site directed mutagenesis.

The post will attract a competitive salary and excellent benefits including 5 weeks holiday, life assurance, sick pay and pension scheme.

Please write with full details to: Mr. K. Tollett, Wellcome Biotechnology Limited, FMD Division, Ash Road, Pirbright, Woking, Surrey GU24 0NQ.

(1905)A

UNIVERSITY OF ZIMBABWE

Applications are invited for the following posts:

PROFESSORSHIP

DEPARTMENT OF CHEMISTRY

Applicants should have a sustained record of recognised international excellence in their specialist area. The successful candidate will be expected to teach and conduct research in either analytical or physical chemistry. Applicants with lesser experience may be considered for a post at a lower level.

LECTURESHIPS/SENIOR LECTURESHIPS/ASSOCIATE PROFESSORSHIPS

DEPARTMENT OF BIOLOGICAL SCIENCE

(3 posts)

(a) Botany (2 posts): Applicants must have a first degree and a PhD and/or research experience in some aspect of plant science. The successful candidates will be required to contribute to undergraduate courses in general botany, plant taxonomy and plant physiology in the Department of Biological Sciences. Applicants from any area of botanical science will be considered but the areas of special preference are those of: (i) Systematic botany, where candidates with experience of the flora of Central and Southern Africa will be of particular interest; (ii) Plant physiology particularly plant growth, water relations or seed physiology.

(b) Nematology/Soil Zoology (1 post): Applicants should be experienced in the general area of Nematology and/or Soil Zoology. Any area of specialisation within these disciplines will be considered but preference will be given to candidates with research experience with plant-infecting or other soil-inhibiting nematodes. The successful candidate should have a first degree in some aspect of biological or agricultural science and a PhD and/or research experience in the appropriate area of specialisation. He/she will be required to contribute to undergraduate courses in invertebrate zoology, parasitology and soil ecology and to appropriate postgraduate courses.

Salary Scales (Under Review): Lecturer: Z$9,936 × 432 — Z$15,120 × 468 — Z$15,588 × 480 — Z$16,548; Senior Lecturer: Z$17,028 × 480 — Z$19,428; Associate Professor: Z$17,508 × 480 — Z$19,908; Professor: Z$19,452 × 612 — Z$20,676 × 840 — Z$23,196.

Appointment on above scales according to qualifications and experience.

Conditions of Service: Both permanent and short-term contracts are offered. Persons who are not Zimbabwean citizens may be appointed only on a short-term contract basis with an initial contract period of two years. Short-term contracts may, in exceptional cases, be extended.

Application forms and further particulars on the above posts can be obtained from the Director, Appointments and Personnel, University of Zimbabwe, PO Box MP 167, Mount Pleasant, Harare, Zimbabwe, or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Closing date for receipt of completed applications forms is 1 June 1984.

(W961)A

HONG KONG BAPTIST COLLEGE

Head of Physics Department

Applicants should have (a) a relevant academic/professional qualification of at least an MA degree, preferably a PhD degree; (b) at least 6 years of post-qualification teaching with a strong basic physics training and preferably further experience in Applied Physics and/or Industrial Research. Duties will include teaching and engaging in research work of related subjects and other departmental administrative work.

Salary Scale: HK$14,660 to HK$19,030 per month by 6 increments. (starting salary will be commensurate with qualifications and experience).

Benefits: Passages to overseas appointee and other fringe benefits including Superannuation (College 15%, appointee 5%), long leave (about 3 months after every 21 months of service), subsidized housing and medical and dental benefits for appointee and family; and where appropriate children's education allowance and passages.

Please send detailed résumé and other relevant credentials to Personnel Office, Hong Kong Baptist College, 224 Waterloo Road, Kowloon, Hong Kong. (W955)A

PLANT BREEDING INSTITUTE

MARIS LANE TRUMPINGTON
CAMBRIDGE CB2 2LQ

MOLECULAR BIOLOGISTS

required to participate in a project funded for 3 years by the Rockefeller Foundation to explore whether viral genes introduced into plant chromosomes can induce resistance to subsequent viral infection. The work will involve the cloning and characterisation of plant viral genes, the insertion of these genes into Ti plasmid vectors and describing the transcription of such genes in transformed plants.

Appointment will be at Higher Scientific Officer (salary £7,149 to £9,561 pa). Qualifications are a first or upper second class honours degree in molecular biology or related subject with at least two years' relevant experience. Reference CYT/164.

SCIENTIFIC OFFICER

required to join the winter wheat breeding team. The principal responsibility of the person appointed wil be to check the uniformity of advanced breeding lines of winter wheat with respect to segregation for important agronomic characters, including disease resistance and grain quality, in preparation for the generation of nucleus pure stocks. These stocks will have to meet statutory requirements for distinctness, uniformity and stability. The team is led by J Bingham Esq FRS.

Appointment will be at Scientific Officer (salary £5,682 to £7,765 pa). Qualifications are a degree in Agricultural Botany or a related subject, preferably with relevant post qualifying experience in experimental work with field crops. Reference CER/168.

Non-contributory pension scheme and pending pay awards apply to both posts.

Applications with curriculum vitae and the names and addresses of three referees for the first post and two for the second should be sent to the Establishment Officer by 24 May 1984, quoting the appropriate reference.

The Plant Breeding Institute is an Equal Opportunities Employer. (1874)A

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF PSYCHIATRY

LECTURESHIP

(Non-Clinical)

Applications are invited from suitably qualified science graduates for appointment to a post of Lecturer in the Department of Psychiatry, available from 1st October 1984. This non-medical post will provide for the permanent appointment in a clinical department of a scientist who will have the opportunity to pursue relevant research in the department's main research interests concerning the functions of some neuropeptides and biogenic amines in brain. Candidates should have several years post-doctoral research experience, particularly in advanced anatomical techniques (eg immunocyto-chemistry, axon tracing) at light and electron microscope level, and some experience of current neurochemical methods employed in the study of aminergic or peptidergic function would be advantageous.

Salary will be at an appropriate point on the Lecturers' Non-Medical scale: £7,190 — £14,125 per annum according to age, qualifications and experience.

Further particulars may be obtained from the Senior Assistant Registrar (Establishments) (FP), The University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU, with whom applications (three copies), together with the names and addresses of three referees, should be lodged not later than 22nd May 1984. Please quote reference N. (1920)A

MULTIPLE MIRROR TELESCOPE OBSERVATORY

STAFF ASTRONOMER

Applications are solicited for the position of staff astronomer at the Multiple Mirror Telescope (MMT) Observatory, a joint facility of the Smithsonian Institution and the University of Arizona, located on Mt Hopkins 60 km south of Tucson, Arizona.

Applicants should have a PhD degree in astronomy or physics and should have extensive experience in optical or infrared astronomical instrumentation. MMTO staff astronomers will be expected to guide the development of state of the art instrumentation for use on the MMT by a large community of users. Astronomical spectrographs, detectors, minicomputers for data acquisition and reduction, (hardware and software), microwave data transmission, and optical and IR interferometric instrumentaiton are areas in which expertise is being sought.

MMTO staff astronomers will spend a large fraction of their time in service to the user community, but are also expected to maintain a vigorous program of astronomical research using the MMTO. Résumés of the applicant's qualifications, along with names of three references, must be received by May 31, 1984 at the following address: Director, MMTO, PO Box 21084, Tucson, Arizona 85720. The University of Arizona and the Smithsonian Institution are Equal Opportunity, Affirmative Action Title IX/Section 504 Employers. (NW449)A

Fermentation/Culture Improvement

International Process Development Group

Pfizer, one of the world's leading pharmaceutical companies, is a multi-national organisation with world-wide manufacturing facilities producing an extensive range of science based products, many of which are made by fermentation.

The International Process Development Group based at Sandwich in Kent is responsible for the development of novel approaches to the fermentation and recovery process used in International Production, covering all major plants outside of the U.S.A. Due to expansion we are currently seeking two new important additions to this group.

A Fermentation Technologist

is required to join the Fermentation Process Development Section. You will join a dedicated team, equipped with a range of modern fermenters, involved in the development, optimisation and scale-up of fermentation processes at both laboratory and plant scale. You should hold a good honours degree in a relevant subject and ideally have a post graduate qualification or previous industrial experience. Our commitment to advancing with technology would make an appreciation of computer control for the optimisation of fermentation processes an advantage.

A Microbiologist/ Biochemist

is required within the Culture Improvement Section. The successful candiate will join an established team using both conventional and recombinant DNA approaches to strain improvement on Streptomyces species. Applicants should have a good honours degree in biochemistry, microbiology or a related subject, preferably with a post graduate qualification and/or relevant industrial or academic expedrience.

These challenging appointments offer a highly competitive basic salary plus a bonus related to personal performance. We also offer generous help with relocation.

Pfizer

We employ some 1400 people, of whom over 250 are graduates, on our Sandwich site which is in pleasant rural surroundings, close to coastal resorts, championship golf courses, the Continent and good sailing waters. We have a thriving Sports and Social Club with first class amenities.

If you are interested in the appointments please send your curriculum vitae, in confidence, to Dr. M.C. Hall, Senior Personnel Officer, Pfizer Limited, Sandwich, Kent CT13 9NJ.

(1955)A

WEST VIRGINIA UNIVERSITY

invites applications and nominations for the position of

DEAN OF THE COLLEGE OF OF AGRICULTURE AND F...ESTRY AND DIRECTOR OF THE AGRICULTURAL AND FORESTRY EXPERIMENT STATION

The Dean is responsible for all educational, research, extension and service programs of the College and Experiment Station. Qualifications include an earned doctorate, a record of academic and research excellence, a firm commitment to the mission of a comprehensive land grant university and ability to communicate effectively with faculty, staff, students and representatives of commodity groups. Demonstrated administrative leadership is desired.

Applications including a résumé, other supporting material and names of three references will be accepted t... July 15, 1984. Nominations and a... ications should be addressed to: Chairman of Search Committee, School of Dentistry, Medical Center, West Virginia University, PO Box 6305, Morgantown, West Virginia 26506-6305.

West Virginia University is an Affirmative Action, Equal Opportunity Employer. (NW497)A

UNIVERSITY OF EDINBURGH

DEPARTMENT OF PHYSICS

RESEARCH FELLOW

Applications are invited for the position of post-doctoral research assistant within the Condensed Matter Group in the Physics Department. The research activities of this group lie largely in the field of phase transitions and critical phenomena, and the successful applicant will be expected to engage in theoretical work in this area, with particular emphasis on Monte Carlo studies using the Edinburgh Distributed Array Processors. Experience in the area of phase transitions and with computing would be an advantage.

The position, which is supported by the SERC, is available for a period of up to three years, and the appointment will be made on the 1A scale at a point commensurate with age and experience.

Applications, giving full details of the candidate's qualifications and research interests, should be sent to: Dr A D Bruce, Physics Department, James Clerk Maxwell Building, Mayfield Road, Edinburgh EH9 3JZ.

Applicants are requested to arrange for letters of support from two referees to be sent directly to the above address.

Please quote reference No 5168. (1928)A

THE UNIVERSITY OF NEW ENGLAND

DEPARTMENT OF ANIMAL SCIENCE

LECTURER/SENIOR LECTURER IN GENETICS

Applications are invited for a tenured position in Genetics. Duties will include undergraduate teaching in Genetics and animal breeding, supervision of honours and postgraduate students and research.
The applicant should have a broad knowledge of Genetics, with particular expertise in quantitative genetics and its applications in animal breeding, some preference will be given to applicants with research experience in theoretical quantitative genetics. Applicants should have a science, agriculture or veterinary degree and appropriate postgraduate qualifications. The Department is well-equipped for Genetic research and is responsible for all Genetics teaching in the University.
Appointment to commence as soon as possible.

Further information may be obtained from the Head of Department, Professor J.S.F. Barker, by writing or telephone (067) 73 2225.

Salary: Lecturer: A$23,394-A$30,734
Senior Lecturer: A$31,390-A$36,585
Closing Date: 4th June, 1984
Position No: 468
The appointment will be to the permanent staff but the University reserves the right to make the appointment probationary where it considers this appropriate.
Other conditions include superannuation, assistance with travel and removal expenses and with buying or building a house in Armidale.

Applications including the names and addresses of three referees and stating the position number should be sent to the Staff Officer, University of New England, Armidale, N.S.W. 2351, Australia, prior to the closing date. Applicants should forward a copy of this advertisement to their referees and ask them to send their reports direct to the Staff Officer, Mr. B.G. Turner, marking their envelopes "Private and Confidential" and quoting the position number, before the above closing date so as to expedite the appointment process.

Armidale NSW 2351, Australia (W962)A

Kumpulan Guthrie Sendirian Berhad, a leading Malaysian Plantation Group with diversified interests

Requires

BIO-TECHNOLOGIST
(TISSUE CULTURE SCIENTIST)

Responsibilities: The successful applicant will be responsible for developing tissue culture techniques for oil palm and other tropical plantation crops for large scale commercial production.

Qualifications: Applicants should have a PhD or Masters Degree in Biological Science or a related discipline to tissue culture. A minimum of 3-5 years experience in developing tissue culture techniques is required preferably in oil palm or other plantation crops.

Applications: Applications must include photocopies of all degrees, reference letters from all previous employers and a full curriculum vitae.

Address applications to:

The Associate Director, Personnel
Kumpulan Guthrie Sendirian Berhad
P O Box 12516, Kuala Lumpur
MALAYSIA. (W944)A

ETH ZÜRICH

The Swiss Federal Institute of Technology in Zurich has an opening for the faculty position of

PROFESSORSHIP in EXPERIMENTAL ATMOSPHERIC PHYSICS

The teaching duties of the new professor will include advanced courses in atmospheric physics for science graduates and basic meteorology courses for engineering and science students. In research, emphasis is to be placed on experimental (including field measurement) work in basic or applied atmospheric physics in one (or more) of the following areas: boundary layer processes, cloud physics, regional circulation systems, aerosols and air pollution problems.

The successful applicant will have gained considerable scientific experience after university graduation and demonstrated ability to perform high quality research. He will be prepared to teach at all university levels and to cooperate both with colleagues within the university and with other institutions.

Applications, with curriculum vitae and list of publications, should be submitted before June 30, 1984, to the President of the Swiss Federal Institute of Technology, Prof. H Ursprung, ETH-Zentrum, 8092 Zurich. (W963)A

BRUNEL UNIVERSITY
Faculty of Mathematics and Science
Department of Applied Biology

'NEW BLOOD' LECTURESHIP

This post is in a department where the research interests are mainly concerned with fundamental biomedical science. The successful applicant will have already demonstrated substantial research potential in this area with post-doctoral experience in cell biology. It is envisaged that he/she will form a link between two major research groups: the Cell Biology Unit (Dr Roger Dean) with major interests in macrophages, proteolysis and the biological effects of free radicals; and the Parasite Immunology Unit (Professor Roland Terry) with major interests in the immunoconfusion accompanying some parasitic infections and other immune evasion mechanisms. In the first instance the appointee will probably be engaged in characterising the disordered mononuclear phagocytes which are present in certain experimental parasitic infections. The person appointed will be expected to teach appropriately in the Cell Biology sections of the enhanced undergradute degree courses in Applied Biology and Applied Biochemistry and also to contribute to the teaching of the postgraduate course in Applied Immunology.

Applicants must be under 35 years on 1 October 1984 and not presently occupying a UGC funded teaching post. Salary will be in the Lecturer scale £7,190 – £14,125 per annum, plus £1,186 per annum London Allowance, with USS benefits.

Requests for additional information and to visit the Department should be made to Mrs Ann Johnston, Department of Applied Biology, Tel: Uxbridge (0895) 37188 ext 646.

Letters of application, accompanied by full curricula vitae and the names of three referees, should be addressed to the Personnel Secretary, Brunel University, Uxbridge, Middlesex UB8 3PH, by 25 May 1984.

Brunel University is an Equal Opportunity Employer. (1882)A

RESEARCH Associate position in molecular endocrinology for a physician/scientist with experience in insulin receptor biosynthesis, processing autophosphorylation mechanisms and clinical abnormalities. Send C vitae and three references to: Donald F Steiner, CLSC 739 Department of Biochemistry, 920 E 58th St, Chicago, IL 60637. (NW499)A

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
MEDICAL SCHOOL
QUEENS MEDICAL CENTRE
RESEARCH ASSISTANT
(readvertisement)

Applications are invited for a Wellcome Trust Funded Research Assistantship to carry out a study of the effect of histamine agonists and antagonists on the release of catecholamines and indoleamines in the CNS.

Experience in the methods of HPLC or neurotransmitter release would be desirable.

The appointment is available from August 1984, for one year, in the first instance, renewable for a further two years with a salary in the range of £6,310 to £7,190 per annum.

Enquiries may be made to Dr S J Hill or Dr R Mason on (9692) 700111 ext 3525. Two copies of a letter of application and a curriculum vitae, naming two referees, should be sent as soon as possible to Dr S J Hill/Dr R Mason, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH. (1891)A

POSTDOCTORAL RESEARCH ASSOCIATE POSITION

available immediately to study the Biochemistry of Exocytosis in the Chromaffin Cell. Protein phosphorylation, calcium-binding proteins, and membrane interactions. See J C B 91: 247 and J B C 258: 14664.

Send Curriculum Vitae and names of references to Dr Carl E Creutz, Department of Pharmacology, University of Virginia, Charlottesville, Virginia 22908. An Equal Opportunity/Affirmative Action Employer. (NW501)A

UNIVERSITY OF GLASGOW
DEPARTMENT OF GENETICS
POSTDOCTORAL POST

A Postdoctoral position is available in the Department of Genetics for years from October 1984, to work with Dr Howard Jacobs on the Isolation and Characterisation of Nuclear-coded Mitochondrial Genes from Mammalian Cells. This research is funded by an MRC Project Grant, and salary will be at the appropriate point on Range 1A of the scales for Research and Analogous Staff, plus USS benefits.

Applicants should be familiar with somatic cell genetics and/or recombinant DNA technique.

Applications, including full curriculum vitae and the names of two referees, should be sent to Dr H T Jacobs, Department of Genetics, University of Glasgow, Glasgow G12 8QQ before 31st May 1984.

In reply please quote Ref No 5242M. (1903)A

INSTITUTE OF TERRESTRIAL ECOLOGY
RADIOCHEMIST

A Radiochemist is needed to join a small team, looking into the distribution of radionuclides in the environment. The appointee will be based at the Institute's Merlewood Research Station, Grange-over-Sands, Cumbria and will be responsible for the radiochemical laboratories and counting instruments, for the development of radiochemical techniques and, with assistance, for the determination of alpha and gamma emitters in ecological materials. There will be opportunity to contribute to the research programme.

QUALIFICATIONS AND EXPERIENCE: The minimum qualification for the post is an honours degree. Previous experience is essential.

SALARY: The appointment will be to Higher Scientific Officer/Senior Scientific Officer grade with a salary scale of £7,149 — £9,561 for HSO or £8,970 — £11,476 for SSO. Starting salary will be according to age, qualifications and experience.

The Natural Environment Research Council is not a government Department but conditions of service are similar to those of the Civil Service.

Application forms and further details are available from: Establishment Section, Institute of Terrestrial Ecology, 68 Hills Road, Cambridge CB2 1LA, (Tel: 0223 69745).

Please quote reference number 229. The closing date for applications is 18 May 1984. (1912)A

Natural Environment Research Council

THE UNIVERSITY OF CALGARY
NEUROANATOMIST

Applications are invited for a NEUROANATOMIST in the Department of Anatomy and the Neuroscience Research Group in The University of Calgary, Faculty of Medicine. Appointment at Assistant or Associate Professor, salary on University scale will be determined according to experience and qualifications. Requires MD or PhD, at least two years post-doctoral research training, evidence of a productive and fundable neuroanatomical research program, and experience teaching medical and graduate neuroanatomy. Primary duty will be to establish and conduct an original, independent research program of interest to a large and diverse interdisciplinary neuroscience group. Teaching in medical and graduate neuroanatomy will also be required. The position to be funded by successful competition for a Scholarship from the Alberta Heritage Foundation for Medical Research.

In accordance with Canadian Immigration requirements, this advertisement is directed to Canadian citizens and permanent residents.

Applications must be received by May 31, 1984, with appointment to be effective in 1985.

Please send a curriculum vitae, statement of research interests and career goals, and the names of three referees to: Dr Gregory E Lucier, Chairman, Neuroanatomy Search Committee, Department of Medical Physiology, The University of Calgary, Faculty of Medicine, 3330 Hospital Drive NW, Calgary, Alberta T2N 4N1. (NW494)A

GLASSHOUSE CROPS RESEARCH INSTITUTE
CROP PROTECTION DIVISION
BIOTECHNOLOGICAL APPROACHES TO STRAW UTILIZATION

Two vacancies are to be financed by the Agricultural Genetics Company for 2 years with possible extension.

1 A Microbiologist/Plant Scientist (grade HSO I/SSO) is required to isolate microbial inoculants to hasten straw biodegradation. Small-scale testing of their efficacy under laboratory conditions will be undertaken.

2 A Microbiologist/Soil Scientist (grade HSO II) is required to produce and formulate existing microbial inoculants for production on a scale suitable for field-scale testing.

Qualifications required are first or upper second class honours degree with 2-4 years relevant post-qualifiying experience for HSO I/SSO grade and a degree or HNC in a relevant subject with at least five post-qualifying years relevant practical experience for the HSO II grade.

Salary £7,149 — £9,561, HSO grade; £8,970 — £11,476 SSO Grade, depending upon qualifications and experience. Non-contributory superannuation scheme. Annual leave 22 days.

Application forms and further details from Administrative Assistant, Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, W Sussex BN16 3PU.

Closing date for applications — 18 May 1984. (1933)A

Pfizer Centre de recherche

Cadre biochimie analytique

Le centre de recherche PFIZER recrute pour renforcer son département biochimie un **cadre biochimie analytique**.

Le poste nécessite la maîtrise des techniques analytiques (HPLC, GLC) et de bonnes connaissances en chimie analytique, en pharmacocinétique et métabolisme des médicaments.

L'encadrement d'un groupe de techniciens, la mise au point de techniques, la préparation des rapports nécessitent une **expérience de quelques années**. Une bonne connaissance de l'**anglais** est impérative.

Lieu de travail : **AMBOISE** (37),

Adresser C.V., photo et prét. au Chef du Personnel - **Laboratoires PFIZER** - B.P. 109 - 37401 AMBOISE Cedex FRANCE. (W953)A

MEDIA 84

MERCK SHARP & DOHME-CHIBRET

SUBSIDIARY OF MERCK AND CO.

seeks for a

GROUP LEADER (PHARMACOLOGY)

MSD market Timoptic®, the market leader in the treatment of glaucoma. The commitment of MSD Research Laboratories to ocular research has been reflected in the reecent construction of an ultramodern research facility located in an attractive region in France.

We are seeking a Group Leader for our Department of Pharmacology. The person will be responsible for the running of a very well equipped laboratory and his (her) duties will include originating and conducting novel research programmes.

The successful candidate will possess a broad experience in both autonomic and cardiovascular pharmacology. Applicants are required to have a Ph.D or equivalent with 2 years or more post-doctoral experience and an established research record, as demonstrated by a significant bibliography of publications.

The salary will be appealing to candidates of a high calibre and career prospects are good. A working knowledge of French is desirable but not essential. However, applicants should be willing to learn the language, for which facilities will be provided.

MSD
MERCK SHARP & DOHME CHIBRET

Please send C.V. together with list of publications and/or other scientific accomplishments to : Direction du Personnel, MERCK SHARP & DOHME-CHIBRET, RESEARCH LABORATORIES, Division of Merck & Co. Inc., Route de Marsat, 63203 RIOM Cedex - France. (W947)A

AGRICULTURAL AND FOOD RESEARCH COUNCIL
FOOD RESEARCH INSTITUTE
Microbiology Division

Post No. 83/26 SCIENTIFIC OFFICER/HIGHER SCIENTIFIC OFFICER

A MICROBIAL GENETICIST is required to work in a group studying the genetics of methanogenic bacteria. The long term objectives of the group are to develop gene transfer systems, so that strains of methane producing bacteria of improved efficiency in anaerobic digestion processes may be produced.

Methanogens are rigorous anaerobes which can only be cultured in conditions of strict anaerobiosis. Genetics methods are not yet established and techniques applicable to Eubacteria may not be directly applicable to these Archaebacteria. The immediate aims are to produce genetically marked strains, to isolate potential vectors and to establish techniques for generating viable protoplasts.

Qualifications:
First or upper second class honours degree in a relevant subject, with at least 2 years relevant post-qualifying research experience in microbial genetics for appointment at Higher Scientific Officer level, and preferably a PhD.

Nutrition and Food Quality Division

Post No. 84/1 SCIENTIFIC OFFICER

This post is one of two such posts in the Intake Measurement Group. The person appointed will be concerned with studies of food intake using a variety of techniques and the preliminary processing of data from these studies. The post calls for an interest in, and an aptitude for, working with people and some dietetic experience is desirable. The studies will be primarily conducted in the Norwich area; a valid driving licence is necessary.

Qualifications:
A degree in Nutrition of Dietetics with some dietetic experience.

Salary scales: Scientific Officer: £5,682 – £7,765. Higher Scientific Officer: £7,149 – £9,561.

Non-contributory superannuation scheme.

The Agricultural and Food Research Council is an Equal Opportunities Employer.

Further particulars and application forms from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA, quoting the appropriate reference number. Closing date: 18 May 1984.

(1925)A

BIOTECHNOLOGISTS!

Are you reading Bio/Technology, the International Journal for Industrial Biology?

For a free sample copy of this new monthly Journal from the Publishers of *Nature*, please write or phone:

Mirta Soto
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15 East 26th Street
New York 10010
USA
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Felicity Parker
Bio/ Technology
Houndmills Industrial Estate
Brunel Road, Basingstoke
Hampsire, UK
Tel.: (0256) 29242

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL RESEARCH ASSISTANT IN BACTERIAL MOLECULAR BIOLOGY

Applications are invited for research on the genetic dissection of E.coli RNA polymerase, a project involving standard molecular biological techniques.

This Grade 1A position, supported by the MRC, is initially for one year, extendable for a further two years, with a starting salary of £7,190 per annum including USS benefits.

Applicants possessing a 2(i) or higher in an appropriate degree should send a curriculum vitae and the names of two referees to Dr Robert E Glass, Department of Biochemistry, Queen's Medical Centre, Nottingham, NG7 2UH (Telephone: 0602 700111 extension 3395) from whom further information may be obtained. (1918)A

RESEARCH Associate — perform research using Nuclear Magnetic Resonance to investigate DNA conformations and effects of cancer related drugs on biological systems. Drug-DNA interactions using 2-dimensional NMR. Deuterium NMR studies of selectively deuterated nucleotides. Protein-inhibitor interaction. PhD required in chemistry. 3 years research experience with demonstrated ability in NMR relaxation methods, deuterium NMR of proteins and biopolymers, 2-dimensional NMR techniques and working knowledge of NMR instrumentation required. 40 hrs/wk, $23,000/yr. Location: Bethesda, MD. Send résumé to Maryland State Job Service, 1100 North Eutaw Street, Baltimore, MD 21201. Refer to Job Order Number 0481337. (NW503)A

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF ZOOLOGY
POSTDOCTORAL RESEARCH ASSISTANT

A neurophysiologist is required to work with Dr. J.B. Messenger on a project, funded by SERC, investigating the neural correlates of colour change in cephalopod molluscs. Prior knowledge of cephalopods is not necessary but experience in intracellular recording techniques is essential and familiarity with iontophoresis would be an advantage.

The successful candidate would have to spend part of the year at laboratories in Plymouth and in France (Banyuls). Tenable from 1 October 1984 for 3 years. Initial salary £7,190 — £8,530 a year on Range IA.

Enquiries to Dr. J.B. Messenger, Department of Zoology, the University, Sheffield S10 2TN to whom applications, including the name of two referees, should be sent as soon as possible. Quote ref: R74/G. (1876)A

PLANT BREEDING INSTITUTE
Maris Lane, Trumpington, Cambridge. CB2 2LQ
PLANT PATHOLOGIST

required to work on powdery mildew of wheat, with particular reference to the development of host resistance and strategies of disease control.

Appointment will be at Higher Scientific Officer (salary £7,149 to £9,561 pa) or Senior Scientific Officer (salary £8,970 to £11,476 pa).

Qualifications are a First or Upper Second Class Honours Degree in an appropriate biological subject, and at least 2 years' postgraduate experience in plant pathology or related research for HSO and 4 years for SSO.

Applications, with curriculum vitae, and the names and addresses of three referees, should be sent to the Establishment Officer by 18 May 1984. Please quote reference Path/171.

Further particulars available on request. The Plant Breeding Institute is an Equal Opportunities Employer. (1932)A

UNIVERSITY OF BATH
SCHOOL OF BIOLOGICAL SCIENCES

Applications are invited for a
POST-DOCTORAL APPOINTMENT

to start on October 1st, tenable for 3 years, to work on an SERC funded project investigating the influence of opioid substances on endocrine function in fishes.

Applicants should hold a PhD and have experience in hormone assays and/or histology.

Salary up to £8,530 pa according to qualifications and experience.

Application forms from the Personnel Officer, University of Bath, Bath, BA2 7AY, quoting ref no: 84/60. Closing date: 21.5.84. (1911)A

CHARING CROSS HOSPITAL MEDICAL SCHOOL
(University of London)
RESEARCH ASSISTANT

Applications are invited for a three year post funded by the Wellcome Trust to work with Dr A K Allen in the Department of Biochemistry on the structure, function & Biosynthesis of cell surface glycoproteins of Trypanosoma cruzi and related protozoa. Previous experience of work on membrane glycoproteins is preferred, but experience of parasitic protezoa is not essential.

Starting salary between £6,310 and £7,630 plus £1,186 London Allowance according to age and qualifications.

For application forms & further details please write to Dr Allen, Department of Biochemistry, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 8RF, within 2 weeks of the appearance of this advertisement. (1893)A

DIVISION OF RESEARCH RESOURCES
NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
DEPARTMENT OF HEALTH AND HUMAN SERVICES

The National Institutes of Health (NIH), Division of Research Resources (DRR) invites nominations and applications for the position of Health Scientist Administrator (Biomedical Sciences), GM-601-14 in the Biotechnology Resources Program Branch (BRP).

This is a Civil Service position with a salary range of $42,722 to $55,538 per annum.

The Biotechnology Resources Program Branch is responsible for the administration of a program currently supporting resource centers which serve as regional and national resources for the acquisition, development and maintenance of ultrasophisticated technologies including instruments, and application of the technologies to study biomedical research problems. The Health Scientist Administrator is responsible for the administration of assistance and procurement awards to the centers. The administrator conducts visits to grantee or potential grantee institutions, plans workshops, symposia and other *ad hoc* meetings, and serves as Executive Secretary of the Biotechnology Resources Review Committee.

Applicant must possess research experience in biomedical computer sciences or biomedical engineering, and meet the minimum requirements for Health Scientist Administrator:
(a) Minimum educational requirements requires a Ph.D. (or equivalent) in a academic field of science allied to health or health related research, *plus*
(b) One year of experience in research program administration in which professional judgments require the application of scientific knowledge of health or health related research to plan, review, and evaluate research projects, and to access accurately the significance of results and progress accomplished.

Further information concerning the position and qualification requirements may be obtained by contacting Ms. Pathenia A. Wiggins at (301) 496-1524. Applications (Personal Qualifications Statement, SF-171) accompanied by a current curriculum vitae and bibliography should be submitted to Ms. Wiggins at the DRR Personnel Office, National Institutes of Health, Building 31, Room 5B06, 9000 Rockville Pike, Bethesda, Maryland 20205. Applications should be post marked no later than May 31, 1984.

Applications from women and minority group members are especially solicited.

THE NATIONAL INSTITUTES OF HEALTH IS AN EQUAL OPPORTUNITY EMPLOYER.

(NW498)A

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF APPLIED MATHEMATICS

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above three year appointment commencing 1 October 1984 at an initial salary in the range £7,190 to £7,630 per annum, plus USS.

The post is funded by an SERC Research Grant and will involve the theoretical study of radio frequency heating of magnetically confined plasmas as part of a programme of research being carried out in collaboration with UKAEA Culham Laboratory. Some knowledge of plasma physics or a related discipline and the use of computers would be an advantage.

Further particulars may be obtained from the Establishments Officer, The University, College Gate, St Andrews, Fife, KY16 9AJ, to whom applications (two copies preferably in typescript), including c v, list of publications and the names of two referees should be sent to arrive not later than 25 May 1984. (1948)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF BIOCHEMISTRY

ELECTROCHEMIST/ BIOCHEMIST

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited from suitably qualified individuals to work with Dr P R Rich on a project funded by the Venture Research Unit of British Petroleum Company p.l.c. The work will involve the application of electrochemical and spectrophotometric techniques to aspects of biological electron transfer processes, particularly those of photosynthesis and respiration. An electrochemist with an interest in biological systems, or a biochemist with a chemical background, would be most suitable.

The post is available immediately for up to three years with a starting salary of £8080 (under review) according to age and experience. Further details of the project may be obtained from Dr Rich. Applications containing a curriculum vitae and the names of two referees should be sent as soon as possible to The Superintendent, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW. (1946)A

SCIENTIFIC EDITOR

The Society for General Microbiology has vacancies for persons to assist in the editing and production of its scientific publications which include the *Journal of General Microbiology* and the *Journal of General Virology*. An interest in scientific publishing and information exchange is essential and some previous publishing experience would be an advantage. Applicants should have a degree in microbiology, biochemistry or a related science, a good command of English and a keen eye for detail. Starting salary will be in the range £6,310 – £7,190 on a scale rising to £9,875 (linked to University Administrative Grade IB). The person appointed would be expected to work in Reading and to live in the neighbourhood.

Applications in your own handwriting, together with a curriculum vitae and the names of two referees, should be sent to Dr C. Sinclair, Harvest House, 62 London Road, Reading, Berkshire RG1 5AS, from whom further details may be obtained on request.

(1909)A

THE UNIVERSITY OF WISCONSIN – MADISON

CELLULAR/MOLECULAR BIOLOGIST

invites applications for a tenure-track faculty position in the general area of structural cellular or molecular biology beginning on or after 1 January 1985. The appointment could be made at the Professor, Associate Professor or Assistant Professor level, depending on the qualifications of the candidate. The candidate would be expected to carry on an active research program and serve as Director of the High Voltage Electron Microscope (HVEM) Laboratory or, in the case of an appointment at the Assistant Professor level, be prepared to assume the Directorship in the future. The HVEM Laboratory is a National Biotechnology Resource providing specialized instrumentation for biomedical structural research including a 1 MeV electron microscope, facilities for electron crystallography, and with plans for the addition of low-voltage scanning electron microscopy and video-enhanced light microscopy. The candidate's research should be directed at a fundamental biological or biomedical problem and might employ, for example, electron crystallography, image analysis of macromolecular complexes or analysis of cell structure. Depending on the background and research area of the candidate the appointment would be entirely or in part in the Department of Anatomy, Biochemistry, Oncology, Pathology, Zoology or in the Laboratory of Molecular Biology. Teaching responsibilities would be determined through the appointing Department.

Applicants should submit, by 1 July 1984, a curriculum vitae, statement of research plans and names of three references to: Professor Gary G. Borisy, Chairman, Laboratory of Molecular Biology, 1525 Linden Drive, University of Wisconsin, Madison, Wisconsin 53706.

An Affirmative Action/Equal Opportunity Employer. (NW496)A

BRITISH GEOLOGICAL SURVEY

(Formerly Institute of Geological Sciences)

COMPUTER CO-ORDINATOR

The Survey has a vacancy for a Computer Co-ordinator at its Edinburgh offices where a PRIME 550 computer is operated on behalf of the Department of Energy as a satellite of a master installation in London and where a data base related to geological exploration of the UK Continental Shelf is currently being installed.

The successful candidate will be responsible to the Hydrocarbon Data Administrator for:

1. **Installation**
   Support, security, maintenance and documentation of the system.
2. **Staff**
   Management of the operator(s)
3. **Programming**
   (a) systems: to optimise and enhance usage of the installation.
   (b) Applications: implementation, modification and support of digitising and graphics program packages.

Applicants should have a degree in a relevant subject and at least 5 years computing experience.

The successful candidate will join at Senior Scientific Officer level (£8,970 – £11,476 pa). Salaries are under review.

The appointment will be for a period of three years after which service in NERC Computing Service may be possible.

NERC is not a government department but conditions of service are similar to those of the Civil Service.

For further details and an application form, write to recruitment Section, British Geological Survey, Keyworth, Nottingham NG12 5GG.

Closing date for receipt of completed application forms is 17 May 1984. Please quote reference SS/ CI/ GT/ 1.

Natural Environment Research Council

(1883)A

POSTDOCTORAL POSITION

available 1 May 1984 for a

CRYSTALLOGRAPHER

interested in the determination of linear polymer structures. Research involves the quantitative use of electron diffraction intensity data from microcrystalline preparations for crystal structure analysis and affords the opportunity to learn unusual aspects of diffraction physics not frequently encountered in X-ray crystallography. Familiarity with X-ray crystal structure analysis and computing is most desirable and some knowledge of electron microscope operation would be useful, but is not essential. Initial appointment is for one year with extension negotiable. Salary is ca $15,000/annum.

Applicants should send curriculum vitae and three references to: Dr D L Dorset, Electron Diffraction Department, Medical Foundation of Buffalo, Inc, 73 High Street, Buffalo, New York 14203.

(NW500)A

MEDICAL RESEARCH COUNCIL

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

Division of Immunology

Applications are invited for a postdoctoral position in the Division of Immunology to work with Dr G.G.B. Klaus on early events in the activation of B lymphocytes by various agents. The appointment will be for 3 years from 1st October 1984. Experience in tissue culture and/or biochemistry would be advantageous.

The salary will be in the range £8530 – £10250 per annum plus £1186 London allowance, with superannuation provision.

Applications should include a curriculum vitae and the names of two referees and should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA before 8th June 1984 quoting ref: IMM/10/H.

(1936)

CAMBRIDGESHIRE COLLEGE OF ARTS AND TECHNOLOGY

PRINCIPAL LECTURER IN GEOLOGY

(Head of Geology)

Required to lead the Geology Section within the Department of Science.

Applicants should possess a PhD in Geology or a related subject, be experienced in teaching Geology to honours degree level, and be active in research.

The ability to teach one or more of the following to honours degree level will be a distinct advantage: economic geology, geophysics, engineering geology, other applied or industrial aspects of geology.

Salary (under review): £12,519 – £13,938 (Bar) – £15,744.

Details and forms, to be returned by 18th May 1984, from Administrative Assistant, Department of Science, CCAT, East Road, Cambridge CB1 1PT. Tel (0223) 352992.

(1939)A

MEDICAL RESEARCH COUNCIL

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

DIVISION OF BIOCHEMISTRY

Applications are invited for a postdoctoral position in the Division of Biochemistry to work with Dr N. M. Green, FRS. The project is in the field of protein chemistry and applicants should preferably have some experience in organic chemistry. The aim of the project will be to develop new photochemical reagents for labelling and identifying functional sites of the Ca^{++} transporting ATPase of sarcoplasmic reticulum and related proteins. Parallel work on crystallisation is also envisaged. The salary will be in the range £8,530 – £10,250 per annum plus £1,186 London allowance, with superannuation provision.

Applications should include a curriculum vitae and the names of two referees and should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA before 31st May 1984 quoting ref: BCH/6/M. (1930)A

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF APPLIED MATHEMATICS

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above three year appointment commencing 1 October 1984 at an initial salary in the range £7,190 to £7,630 per annum, plus USS.

The post is funded by an SERC research grant and will involve the theoretical study of radio frequency heating of magnetically confined plasmas as part of a programme of research being carried out in collaboration with UKAEA Culham Laboratory. Some knowledge of plasma physics or a related discipline and of the use of computers would be an advantage.

Further particulars may be obtained from the Establishments Officer, The University, College Gate, St Andrews, Fife KY16 9AJ, to whom applications (two copies preferably in typescript), including C.V., list of publications and the names of two referees, should be sent to arrive not later than 25 May 1984.

(1901)A

UNIVERSITY COLLEGE LONDON

"NEW BLOOD" LECTURESHIP

EXPERIMENTAL PATHOLOGY

Applications are invited for the above Lectureship in the Department of Experimental Pathology under the UGC's "New Blood" initiative. The post is available from 1st October 1984 and candidates should normally be aged 35 or under.

Applicants should be in the position of initiating and carrying out a research programme in the field of the transport and chemical processing of viral proteins within cells.

Salary in range £7,190-£14,125 per annum (under review) plus £1,186 per annum London allowance.

Applications (no forms), including a full cv and names and addressed of two referees, should be sent to: Assistant Secretary (Personnel), University College London, Gower Street, London WC1E 6BT by no later than 8 June 1984. (1950)A

INSTITUTE OF CANCER RESEARCH

There is a vacancy for a

POST-DOCTORAL SCIENTIST

to work in the Institute's Radiotherapy Research Laboratories at Sutton, Surrey. The successful applicant will join a team of scientists who are investigating the radiobiology of human tumours. This is an exciting area of research at the present time in which there are good prospects for increasing our understanding of the clinical response of cancer to radiation treatment. The work will involve studies on cell suspensions, multicellular spheroids, and human tumour xenografts. The modification of radiosensitivity by drug treatment will be an important aspect of the work. Experience in radiobiology and tissue culture would be an advantage. Appointment to 31.3.86 in first instance. Salary in scale £7,190 – £9,425 pa, plus London Allowance of £1,186 pa.

Applications in duplicate with the names of two referees to the Personnel Officer, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref: 300/G/48. (1923)A

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LONDON OFFICE
Keith Walesby
4 Little Essex Street
London WC2R 3LF
Tel: 01 240 1101
Telex: 262024

NEW YORK OFFICE
Miss Gene Fein
15 East 26 Street
New York, NY 10010
Tel: (212) 689 5900

TORONTO OFFICE
Peter Drake Associates
32 Front Street West
Suite 200, Toronto,
Ontario M5J 1C5
Tel: (416) 364 1623

Mechanisms of Stimulus Response Coupling In Platelets
A Symposium
27th & 28th September 1984
Institute of Psychiatry, De Crespigny Park, London SE5, United Kingdom

Platelet receptors
D. Barnett UK, N.J. Cusack UK, N. Crawford UK
T. Detwiler USA, P.P.A. Humphrey UK, R.L. Jones UK
G. Marguerie France, S. Shapiro USA, D. Tuffin UK
B.J.R. Whittle UK

Control of calcium mobilisation
F. Carey UK, E.F. Luscher Switzerland, D.E. MacIntyre UK
R.H. Michell UK, T.J. Rink UK, E. Salzman USA
M.C. Scrutton UK, J. Westwick UK

Phosphorylation of platelet proteins and nucleotide metabolism
M. Castagna France, J.M. Gerrard Canada, R.J. Haslam Canada
H. Holmsen Norway, A.M. White UK

Products of phospholipid metabolism
M. Chignard France, M.A. Lagarde France, G.P. Lewis UK
S. Moncada UK, J.B. Smith USA, J.R. Vane UK
R.B. Wallis UK

Functional platelet responses
E.M. Bevers Netherlands, G.V.R. Born UK, J. Caen France
V. Ellis UK, J.L. Gordon UK, R.M. Hardisty UK
V.V. Kakkar UK, D.A. Lane UK, M.F. Scully UK

This symposium will provide a forum for critical assessment of recent developments in understanding of the molecular events in platelet stimulus response coupling. The programme has been devised to provide a comprehensive and structured view of current developments. It is hoped that such a symposium will suggest appropriate strategies in the future development of drugs to modulate platelet function.

The symposium proceedings and discussions will be published promptly. The number of participants will be limited to 250, and registration deadline is 1 August 1984. Address for detailed programme and registration form: Elizabeth Wilson, Administrative Secretary, c/o Dr. J. Westwick, Thrombosis Research Unit, King's College School of Medicine & Dentistry, Denmark Hill, London SE5 8RX, United Kingdom. Tel: 01-274 6222 ext 2442/2444.
(1902)M

ANNOUNCING
The UICC Fukuoka Symposium 1984
on
Fundamentals and Clinical Aspects of Digestive Tract Tumors

Sept 20 — Oct 2, 1984
Fukuoka, Japan

Carcinogenesis, Tumor Characteristics,
New Trends in Cancer Diagnosis,
Long-term Survival and Combined Modality,
Multidisciplinary Approaches to Cancer Prevention
will be the topics discussed by forty-eight
International Invited Lecturers.

Information and Circulars
Kiyoshi Inokuchi, M.D.
Chairman
Department of Surgery II
Faculty of Medicine
Kyushu University
Fukuoka 812
Japan
(W931)M

TENTH EMBO ANNUAL SYMPOSIUM 1984
Control of Transcription in Eukaryotes

24—27 September 1984

EMBL Heidelberg (F.R.G.)

Invited speakers include:

M. E. Akam (Cambridge; V. G. Allfrey (New York); M. Beato (Marburg); P. Borst (Amsterdam); F. Cuzin (Nice); E. M. De Robertis (Basel); A. van der Eb (Leiden); H. Eisen (Paris); D. Gallwitz (Marburg); W. J. Gehring (Basel); F. G. Grosveld (London); I. Grummt (Würzburg); P. Gruss (Heidelberg); L. Hereford (Boston); A. Johnson (San Francisco); C. Kédinger (Strasbourg); W. Keller (Heidelberg); U. Laemmli (Geneva); T. Maniatis (Cambridge, USA); K. Nasmyth (Cambridge); M. S. Neuberger (Cambridge); C. S. Parker (Pasadena); M. Revel (Rehovot); R. G. Roeder (New York); W. Schaffner (Zürich); U. Schibler (Lausanne); W. T. Schrader (Houston); G. Schütz (Heidelberg); A. Sentenac (Gif-sur-Yvette); P. A. Sharp (Cambridge, USA); T. Shenk (Stony Brook); K. Struhl (Boston); G. Tocchini-Valentini (Rome); C. Weissmann (Zürich); N. M. Wilkie (Glasgow); K. R. Yamamoto (San Francisco); M. Yaniv (Paris)

Although the main theme of the Symposium is the control of transcription in eukaryotes, some aspects of RNA processing will also be discussed.

The Symposium will be at the European Molecular Biology Laboratory, Heidelberg, with registration on Sunday, 23 September 1984. Plenary sessions will be held in the mornings and evenings of 24 — 26 September, and the morning and afternoon of 27 September. There will be poster sessions in the afternoons of 24 — 26 September inclusive, to allow participants to present their work. It is also intended to organize informal workshops during the Symposium, the themes of which, depending on the posters received, may include inducible genes, cell-type specific gene expression, promoter organization and the structure of active chromatin.

Applications for participation, including a curriculum vitae, a brief description of current research interests, and a 1-page (DIN A 4) abstract of your poster, if you wish to present one, should be sent to: Dr. J. Tooze, Executive Secretary, EMBO, Postfach 1022.40, D - 69 Heidelberg, F.R.G.

The registration fee, which includes daily transport to the EMBL from Heidelberg during the Symposium, lunches, and the Symposium reception is DM 180, for graduate students DM 90, and for participants from industry DM 360. Participants will be accommodated in hotels in Heidelberg, and a small number of inexpensive rooms will be available in the EMBL Guest House. The registration fee does NOT cover the cost of accommodation.

Applications should reach Heidelberg by 1 June 1984. The facilities available make it necessary to restrict the number of participants to about 220 and, therefore, the Organizing Committee may be obliged to make a selection from the applications received. Those accepted will be notified by 31 July 1984 at the latest. The registration fee should NOT be sent until you have been notified of your acceptance for the Symposium.

Organizing Committee: L. Philipson (EMBL Heidelberg) — Chairman, M. Birnstiel (Zürich), P. Chambon (Strasbourg), R. Cortese (EMBL Heidelberg), H. Pelham (Cambridge), J. Tooze (EMBO). (W715)M

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(W547)L

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CONFERENCES and COURSES

EMBO COURSE

BIOTIN-LABELED DNA in MOLECULAR GENETICS

December 3-8, 1984

Laboratoire de Génétique Moléculaire, Institut Jacques Monod, 2 place Jussieu, 75005 Paris, France.

Programme: A practical and Lecture Course detailing the preparation and use of non-isotopically labelled polynucleotide probes, hybridization procedures, (Southern dot, colony, plaque and *in situ*) will be performed. Other applications of these probes as affinity reagents for the purification of nucleic acids and proteins will be discussed.
The teaching staff will include: David Ward (Yale University, New Haven), Pennina Langer-Safer (Roche Institute, Nutley) and Laura Manuelidis (Yale University, New Haven); and researchers of the host laboratory.
Cost: There will be a $100 registration fee (not covering accommodation or boarding) payable following notification of acceptance.
Applications: A letter of application together with a short Curriculum Vitae and a statement of interests and reasons for applying should be sent to Dr. G. Bernardi before September 30, 1984 at the above address. (W946)C

continued on pages 20 & 22

STUDENTSHIPS

LUTON COLLEGE OF HIGHER EDUCATION
LEA RESEARCH STUDENTSHIP in the Geology of Stratabound Mineral Deposits

Applications are invited from graduates with a first or upper second class honours degree or from undergraduates who are likely to obtain such a qualification in the summer, for a Research Studentship funded by Bedfordshire County Council.

The successful applicant will carry out an investigation of the Palaeozoic iron-ores of Caledonides of North Wales under the direction of Dr W E G Taylor with the collaboration of Dr A V Bromley of Camborne School of Mines.

Full Curriculum Vitae to be returned to Dr Taylor, Luton College of H.E., Park Square, Luton LU1 3JU by Friday 25th May 1984.

(Beds C.C. is an equal opportunities employer) (1879)F

UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST THOMAS'S HOSPITALS
PAEDIATRIC RESEARCH UNIT AT GUY'S
PhD STUDENTSHIP IN MOLECULAR BIOLOGY

Post available for student with very good academic record wishing to investigate, under the supervision of Dr F Giannelli, the gene defects causing deficiency of coagulation factor IX and haemophilia B. Previous project work or experience in molecular biology an advantage.

Full curriculum vitae, with the names of two referees, should be sent to the Administrative Assistant, Paediatric Research Unit, Prince Philip Research Laboratories, Guy's Tower, London Bridge, London SE1 9RT. (1906)F

MEAT RESEARCH INSTITUTE
AFRC — RESEARCH STUDENTSHIP

Applications are invited for a 3 year AFRC Postgraduate Studentship at the Meat Research Institute to study proteinase activities in muscle. The work will include the production of monoclonal antibodies for the development of immunoassays and immunohistochemical location of specific proteinase.

Candidates must hold (or expect to gain) a First or Upper Second Class Honours degree in Biochemistry or Cell Biology and will be expected to register as a Ph.D Student at the University of Bristol.

Applications forms and further details from: The Personnel Officer, Meat Research Institute, Langford, Bristol BS18 7DY. (1935)F

UNIVERSITY OF ESSEX
SERC CASE STUDENTSHIPS

Applications are invited for a CASE Studentship, tenable in the Physics Department and in collaboration with the Allen Clark Research Centre of the Plessey Company to investigate conduction processes in GaAs/(GaAl) As heterojunctions grown by MOCVD methods.

Applicants, who should possess or expect to gain a 1st or 2(i) honours degree in Physics, should send their curriculum vitae (including the names of two referees) to the Departmental Secretary, Physics Department, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, from whom further particulars may be obtained, not later than 31 May 1984 quoting reference N. (1884)F

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF PHYSIOLOGICAL SCIENCES
THE MEDICAL SCHOOL

Applications are invited for an

MRC FUNDED THREE-YEAR PhD STUDENTSHIP

to investigate the origin, composition, physical and biochemical properties of middle ear effusions in chronic secretory otitis media in children. Candidates should have, or should expect to obtain, a First Class or Upper Second Class Honours Degree in Physiological Sciences, Biochemistry or a related subject.

Further details can be obtained from Dr J P Pearson, Department of Physiological Sciences, The Medical School, The University, Newcastle upon Tyne NE1 7RU, to whom letters of application including a curriculum vitae and the names of two academic referees should be sent as soon as possible. (1892)F

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL
DEPARTMENT OF BIOCHEMISTRY
SERC STUDENTSHIP IN MICROBIOLOGICAL BIOCHEMISTRY

Applications are invited from final year students in an appropriate field, who expect to achieve first or upper-second class honours, for an award tenable for three years. The successful applicant will register for a PhD degree and will be involved in a study of the regulation of extracellular toxin formation in Staphylococcus aureus.

Applications giving details of academic record and the names of two referees should be sent to Dr G Coleman, Department of Biochemistry, Nottingham University Medical School, Queen's Medical Centre, Nottingham NG7 2UH. (1942)F

UNIVERSITY OF CAMBRIDGE
THE BIOTECHNOLOGY CENTRE
SERC BIOTECHNOLOGY PhD STUDENTSHIP

Applications are invited from graduates in appropriate disciplines for a research studentship in biosensors commencing in July 1984 and tenable for three years. The project will involve the development of immunologically-sensitised field effect transistors for application in clinical diagnostics and will be under the supervision of Dr C R Lowe.

Applicants must hold, or expect to gain, a first or upper second class honours degree and should send a curriculum vitae together with the names and addresses of two referees to Dr C R Lowe, The Biotechnology Centre, University of Cambridge, Downing Street, Cambridge CB2 3EF. (1937)F

UNIVERSITY COLLEGE OF WALES, ABERYSTWYTH
Welsh Plant Breeding Station
ARFC RESEARCH STUDENTSHIP

Applications are invited for an AFRC Research Studentship tenable from October 1984 to investigate: "The genetic control of self and cross fertility and its manipulation in Lolium"

The successful candidate, who should possess or expect to gain a good Honours degree in Genetics, Botany or an allied discipline, will be registered with the University of Wales for a higher degree.

Applications, including the names of two referees, should be submitted before 1 June 1984, to the Secretary, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB, from whom further particulars can be obtained. (1907)F

CASE STUDENTSHIP UNIVERSITY OF NOTTINGHAM/ICI CORPORATE BIOSCIENCE GROUP

A CASE Studentship is available from 1st October, 1984 to study "Heterologous gene expression in mamary cell lines". This study with transfected genomic clones will be co-supervised by Dr J Windass at ICI and Dr R J Mayer, Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH.

Applications should be sent to Dr R J Mayer at the above address and should be accompanied by the names of two academic referees. (1895)F

UNIVERSITY OF ABERDEEN
INORGANIC SOLID-STATE CHEMISTRY

Applications are invited for the undernoted two CASE studentships. Applicants should hold, or expect to receive, a first or upper second class honours degree. Each studentship will lead to contact with a progressive, expanding company at the forefront of new developments in materials chemistry. Travel and subsistence will be paid for the periods which the student spends in the sponsors' laboratories.

1. Solid-state Oxide Chemistry of Ruthenium and its Compounds (In collaboration with Johnson Matthey, plc, Sonning Common, nr Reading). Ref 1222.

2. Hydrothermal Chemistry of Zirconium Dioxide (In collaboration with Cooksons Group plc, Perrivale, Middlesex). Ref 997.

Additional information can be obtained from the academic supervisor, Prof F P Glasser (Tel 0224-40241 ext 5640) and application forms obtained from the Secretary to the University, Regent Walk, Old Aberdeen AB9 1FX, as soon as possible, but in any event no later than 30 June. (1873)F

STUDENTSHIPS continued

UNIVERSITY OF NOTTINGHAM
MEDICAL SCHOOL
DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
POSTGRADUATE STUDENTSHIP

Applications are invited from those with, or who expect to obtain, a good Honours degree in Physiology, Pharmacology or a closely-related discipline, for an MRC postgraduate research studentship.

The topic of the research will be either (i) the effect of cytoplasmic calcium on glycolysis or (ii) non-invasive respiratory monitoring.

The terms and conditions of the studentship will be those pertaining to MRC Research Studentships.

Applications in writing indicating the preferred topic of research, including a full curriculum vitae and the names of two academic referees, to be sent as soon as possible to Professor A T Birmingham, Head of Department, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH. (1919)F

UNIVERSITY OF LEICESTER
DEPARTMENT OF BIOCHEMISTRY
CASE STUDENTSHIPS

Applications are invited for CASE studentships (Academic Supervisor: Dr. Colin W. Jones) to work on (i) methanol oxidation by methanotrophic bacteria (Cooperative Supervisor: Dr. M.A. Carver, I.C.I.), and (ii) the growth of energetics of Rhizobium (Cooperative Supervisor: Dr. J.W. Drozd, Shell Research).

Applications, enclosing a curriculum vitae and the names of two referees, should be sent to Mrs. J. Dean (Departmental Secretary), Department of Biochemistry, University of Leicester, Leicester LE1 7RH. (1880)F

UNIVERSITY OF KENT AT CANTERBURY
BIOLOGICAL LABORATORY
SERC CASE STUDENTSHIP

Applications are invited from students graduating in a biological science for a three year SERC CASE studentship, commencing in October 1984. The project, in collaboration with ICI, will investigate factors affecting the stability of broad host-range plasmids in bacteria, and will be supervised by Dr M F Tuite and Dr D J Hardman (Kent) and by Dr P Barth and Dr K Powell (ICI).

Further particulars may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, University of Kent, Kent CT2 7NH, to whom completed applications and a curriculum vitae and the names of two academic referees should be submitted by Thursday, 31 May 1984. Please quote ref PG12/84/N. (1886)F

UNIVERSITY OF BRISTOL
DEPARTMENT OF BIOCHEMISTRY
MRC PARTNERSHIP STUDENTSHIP AWARD

Applications are invited (from graduates who have obtained, or are likely to obtain, a first or upper second class degree in Biochemistry) for a 3 year studentship to work on red cell membrane abnormalities associated with disease using monoclonal antibodies as molecular probes.

The studentship will commence in October 1984 and will be carried out under the joint supervision of Dr M J A Tanner, Department of Biochemistry, University of Bristol, Bristol BS8 1TD and Dr D J Anstee, Department of Immunochemistry, South Western regional Blood Transfusion Centre, Bristol BS10 5ND.

Applications (including a brief curriculum vitae and the names of two referees) should be made directly to either Dr Tanner or Dr Anstee. The successful candidate will be registered for the degree of PhD with the University of Bristol.

Applications should be made as soon as possible and not later than June 1st. (1908)F

The Queen's University of Belfast
RESEARCH STUDENTSHIP
Department of Medicine

This Medical Research Council Research Studentship is tenable for the academic year 1984/85, and renewable for up to three years. The candidate who should hold, or be expected to achieve, a good honours degree at Summer graduation will be expected to register for a higher degree. The research will be related to pancreatic and gut hormones. Applications with full curriculum vitae and the names and addresses of two refrees by 1 June to Professor K. D. Buchanan, Department of medicine, The Queen's University of Belfast, Royal Victoria Hospital, Belfast BT12 6BA, from whom further details are available. (1915)F

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF MICROBIOLOGY
MICROBIAL TECHNOLOGY GROUP
CASE STUDENTSHIP

Applications are invited from students who hold or who expect to obtain a first or upper second class honours degree in Biochemistry, Microbiology or a related subject. The project, in collaboration with the Centre for Applied Microbiology, Porton Down, involves the development of cloning and expression vectors for Bacillus subtilis.

Applications, together with a curriculum vitae and the names of two referees, should be sent to Dr C R Harwood, Department of Microbiology, The Medical School, The University, Newcastle upon Tyne, NE1 7RU, from whom further details may be obtained. (1921)F

ROTHAMSTED EXPERIMENTAL STATION
Harpenden, Herts, AL5 2JQ
PHYSIOLOGY & ENVIRONMENTAL PHYSICS DEPARTMENT
SERC CASE STUDENTSHIP: MATHEMATICAL MODELLING OF THE DRIFT OF AGRICULTURAL SPRAYS

Applications are invited for a SERC CASE Studentship tenable at Rothamsted Experimental Station and Imperial College of Science and Technology to study the drift of agricultural sprays downwind from crops. The project will involve field experiments and the use and adaptation of air pollution dispersion models.

Candidates should hold, or expect to obtain, a First or Upper Second Class Degree in Physics, Meteorology, Applied Mathematics or an Environmental Science.

Applications giving full c v and the names of two referees should be sent to: Dr H A McCartney, Physiology & Environmental Physics Department, Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ by 1st June 1984 quoting Ref: 557.

We are equal opportunities employers. (1938)F

OXFORD UNIVERSITY RESEARCH LABORATORY FOR ARCHAEOLOGY

Carbon-14 dating using a 2 MV tandem accelerator, thermoluminescence dating, archaeomagnetism: studentship(s) for DPhil research, suitable for physicists, chemists, earth scientists, anticipating a good honours degree.

Details: Dr M Aitken or Dr R Hedges, 6 Keble Road, Oxford OX1 3QJ, telephone 55211. (1900)F

NUTRITIONAL CONSULTATIVE PANEL of the UK Dairy Industry is offering a POST GRADUATE STUDENTSHIP

for work, possibly leading to a higher degree in a British University or Research Institution on a Nutritional subject related to:
MILK AND MILK PRODUCTS IN THE HUMAN DIET
Examples of subjects which the Panel has identified are:

1) the role of calcium in cow's milk.

2) the role of cow's milk in the weaning of human infants.

Other subjects will be considered. Salary will follow MRC guidelines.

Apply in writing, maximum 500 words, before 31st May 1984 to: The Secretary, Nutritional Consultative Panel, Giggs Hill Green, Thames Ditton, Surrey, KT7 0EL, including proposed department where work will be carried out, curriculum vitae and names and addresses of 2 referees. (1877)F

THE QUEEN'S UNIVERSITY OF BELFAST
FACULTY OF AGRICULTURE AND FOOD SCIENCE

Applications are invited from graduates for the following awards tenable in departments of the Faculty of Agriculture and Food Science.
A: NATURAL ENVIRONMENT RESEARCH COUNCIL
1 studentship to be filled by either Department —
(a) Dept. of Agricultural and Food Bacteriology. Topic: Nitrogen fixation by the wild legume — rhizobium association.
(b) Dept. of Mycology & Plant Pathology. Topic: Basidiomycete mycorrhizal associations with turf and pasture grasses.
B: MINISTRY OF AGRICULTURE FISHERIES AND FOOD
2 Studentships awarded —
(a) Dept. of Agricultural & Food Chemistry. Topic: Denitrification in intensively managed grassland swards.
(b) Dept. of Agricultural Zoology. Topic: Potentiation of the effect of natural enemies on cabbage root fly populations.
C: SERC CASE STUDENTSHIPS
Department of Mycology and Plant Pathology available on the following topics:
"Microbial flax retting after glyphosate herbicide spraying" "(in conjunction with the Lambeg Industrial Research Association, Belfast). "Biology of soft rot erwinians on potato leaves" (in conjunction with the Scottish Crop Research Institute, Dundee).
Applicants must hold a First or Upper Second Class Honours degree in botany, biology or microbiology.
Application should be made to the Head of the relevant Department at Faculty of Agriculture and Food Science, Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX. (1913)A

UNIVERSITY OF NOTTINGHAM FACULTY OF AGRICULTURAL SCIENCE
SERC CASE STUDENTSHI

Applications are invited for a th year PhD studentship, commenc in October 1984, for research on soil-plant water relations of reston opencast mining sites. This award held co-operatively with the Natio Coal Board and will form part o multidisciplinary resear programme aimed at improv restoration procedures. Applica should have, or expect to obtain 1984, a first or upper second c honours degree in an appropr science subject, preferably with sc specialism in soil science. As project involves field work, a curr driving licence is essential.

Further details may be obtai from Dr C R Black, Departmen Physiology and Environme Science, University of Nottingh School of Agriculture, Sut Bonington, Loughborough L 5RD, to whom applicatio including a full cv and the name two academic referees should be as soon as possible. (1943)

SANDOZ RESEARCH INSTITUTE

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A post doctoral fellowship in a research group working on the CLONING OF LYMPHOKINE GENES is available for a

CELL/MOLECULAR BIOLOGIST

with interest in the expression and function of lymphokines. Previous experience in molecular biology, immunology and/or gene cloning techniques in bacteria or yeasts will be of advantage.

The successful candidate will find excellent working conditions in well equipped laboratories. The appointment will be for an initial 2-year period. Moving expenses will be paid.

Please send cv, description of scientific experience and names of two referees to

SANDOZ

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Personnel Department
Brunner Strasse 59
A-1235 Wien, Austria

(W939)E

THE UNIVERSITY OF AUCKLAND
Private Bag, Auckland,
New Zealand
POSTDOCTORAL FELLOWSHIPS

Two Fellowships are available for 12 months to be taken during 1984. Those appointed must have successfully completed the requirements for PhD or equivalent degree before taking up the Fellowship. A monthly allowance of NZ$1,805 will be paid.

PSYCHOLOGY Applicants should have had some experience in the use of small computers to control experiments in human perception and cognition. The Fellow will work with Professor M C Corballis on the relations between perception and imagery. Further information, including application forms, is available from Professor M C Corballis, Department of Psychology.

ZOOLOGY Applicants should have skills in analytical natural products chemistry, be able to devote time to the screening of extracts and have the experience to recognise novel compounds such as carotenoids, isonitriles, sterols and didemnins in marine invertebrates. The collection, identification and extraction to yield both lipid and water soluble components and analysis of extracts by TLC, GLC, HPLC, NMR and Mass spectrometry will follow. Experience in marine natural products chemistry would be an advantage. Further information, including application forms, is available from Professor Patricia R Bergquist, Department of Zoology.

(W951)E

CONFERENCES and COURSES

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THE MOLECULAR BIOLOGY OF CANCER

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Please do not telephone. Write, including a brief synopsis of poster subject and its scientific relevance. Poster space is limited, so we suggest you act promptly.

(NW468)C

MASSEY UNIVERSITY
Palmerston North,
New Zealand
DEPARTMENT OF MICROBIOLOGY AND GENETICS
POSTDOCTORAL RESEARCH FELLOWSHIP IN GENE MANIPULATION

Applications are invited for the above position funded jointly by the NZ Forest Service and the NZ Energy Research and Development Committee to work with Dr Roy Thornton on the development of flocculent strains of pentose fermenting yeasts by genetic manipulation. Experience in gene manipulation techniques is essential and an interest in fermentation processes desirable.

The appointment is available from May 1984 or soon after for an initial period of two years, Remuneration will be NZ$25,000 per annum and some finance will be available for relocation expenses.

Further details of the position and of the University, together with conditions of appointment, may be obtained from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University with whom applications close on 2 June 1984. (W958)E

UNIVERSITY OF STRATHCLYDE

Department of Metallurgy

SENIOR RESEARCH FELLOWSHIP

Applications are invited from well-qualified graduates in Physics, Chemistry or Materials Science for a Senior Research Fellowship (tenable for one year in the first instance). The successful applicant will engage in research on plasma deposition of materials under an SERC supported co-operative project with Standard Telecommunications Laboratories. Experience in Plasma-Chemical or Mass-Spectrometric techniques would be an advantage.

Salary on Range IA for Research Staff (£7,190 – £11,615 p.a.). USS benefit.

Applications, with full curriculum vitae, and the names and addresses of three referees should be sent to Staff Office, University of Strathclyde, McCance Building, 16 Richmond Street, Glasgow G1 1XQ by 25 May 1984 (quote ref: R11/84). (1944)E

THE OPEN UNIVERSITY FACULTY OF SCIENCE RESEARCH FELLOWSHIP IN EARTH SCIENCES

Applications are invited for a three year post-doctoral Research Fellowship in Geochemistry. Candidates should have a proven ability to develop rather than simply to use analytical techniques, and will be expected to make a positive contribution in this area. Present research interests in the Department include: the relationship between magma genesis and mineralisation in orogenic areas (N Chile — Bolivia, Central America and Namibia); open system differentation in high level magma chambers; and trace element enrichment processes in the upper mantle as inferred from mantle xenoliths and within plate basalts. Other specific areas of interest are the geochemistry of platinum group elements, trace element mineral analysis by microprobe and INAA, and fluid inclusion studies. The available analytical equipment consists of an ED XRF, two microprobes, INAA, and both solid source and gas mass spectrometers. Applicants are invited to submit a brief outline of their research interests.

The appointment will be for 3 years, from a starting date to be negotiated between the successful candidate and the University.

Applicants should possess a good first degree in geology and PhD in geochemistry. Salary will be on the 1A Scale for Research and Analogous Staff (£7,190 — £11,615 — under reveiw). Application forms and further particulars are available from Dr. J. W. R. Smith, Administrative Assistant (Science) (4727/2), Faculty of Science, The Open University, Walton Hall, Milton Keynes MK7 6AA or telephone Milton Keynes (0908) 652098/653993: there is a 24 hour answering service on Milton Keynes 653868.

Closing date for applications: 1st June. (1941)E

Southampton THE UNIVERSITY

DEPARTMENT OF OCEANOGRAPHY

PHYSICAL OCEANOGRAPHY/ INFRA-RED RADIOMETRY

A postdoctoral fellowship is to be supported by a NERC Remote Sensing Special Topic Award, to study the physics of the sea surface thermal skin and its importance to infra-red radiometry of sea surface temperature. The post is offered on RA scale 1A and will last until at least March 31st, 1987. Applicants should be well qualified physical science graduates with proven research experience in experimental/applied physics or physical oceanography.

Applications by 14th May to: Dr. I. S. Robinson, Department of Oceanography, University of Southampton SO9 5NH, from whom further details are available. (1924)E

THE ROYAL SOCIETY IBM RESEARCH FELLOWSHIP

Applications are invited for an IBM Research Fellowship, for research in computer science and preferably its applications including remote sensing, digital mapping and other forms of computerized data acquisition, storage and dissemination. Tenure in any appropriate university department of research institution in the United Kingdom is for two years from 1 October 1984. Applicants (restricted to citizens of the United Kingdom or the Commonwealth) must have a PhD or equivalent research experience and should preferably be under 33 on 1 October 1984. Current stipend scale: £10,829 ×£466 to £13,159 per annum with superannuation benefits.

Forms obtainable from The Executive Secretary (UMAM), The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, must be returned by 31 May 1984. (1897)E

POST-DOCTORAL Fellowship: A post-doctoral position is available for candidates interested in immunology and with experience in molecular genetics, especially in gene transfer techniques, to work in a laboratory specializing in molecular analysis of cellular interactions in the immune response. Send résumé and letters of reference to: Drs Charles A Janeway, Jr and Donal B Murphy, Department of Pathology, Yale University School of Medicine, New Haven, CT 06510. (NW480)E

THE UNIVERSITY OF SYDNEY PROFESSOR HARRY MESSEL RESEARCH FELLOWSHIP IN PHYSICS

Applications are invited for the above postdoctoral fellowship for research in Physics in the University of Sydney. The following fields of research are pursued in the School of Physics: Plasma Physics (Tokamak), Solar Energy (selective surfaces), Astrophysics (synthesis radio-telescope), Astronomy (Michelson interferometer), High Energy Nuclear Physics (neutrino physics), Environmental Physics (radio telemetry) and Theoretical Physics pertaining to the foregoing experimental topics. Further information may be obtained by writing to the Head of the School of Physics.

The annual stipend shall be within the range for University of Sydney Postdoctoral Research Fellowships (currently $20,164 to $22,513 per annum). The Fellowship is tenable for one year but may be renewed from year to year.

The University reserves the right not to proceed with this appointment for financial or other reasons.

Application forms or further information may be obtained from the Registrar (Scholarships Office), University of Sydney, NSW, Australia 2006. Applications close on 31 May 1984. (W957)E

Continental European Fellowship Awards For Academic Staff

Applications are invited for awards by the CIBA-GEIGY Fellowship Trust for the academic year 1985/86.

Senior CIBA-GEIGY Fellowships

Senior CIBA-GEIGY Fellowships will be awarded to start from an agreed date during the academic year 1985/86. These will be available normally to lecturers, senior lecturers, readers or those who have held comparable permanent teaching positions for a period of at least five years in the field of chemistry, biochemistry, chemical technology, chemical engineering and biology, particularly in its relation to chemistry, at United Kingdom or Republic of Ireland universities, polytechnics, or comparable teaching institutions, who wish to undertake research at an agreed continental European university or technological university.

Awards are made for a period of six to twelve months.

Value of the awards: £9,000 per annum plus travelling expenses. CIBA-GEIGY Fellowships are intended to supplement Fellows' salaries for the period of tenure.

Further details and application forms, which should be submitted before 1 November 1984, can be obtained from The Secretary, The CIBA-GEIGY Fellowship Trust, 30 Buckingham Gate, London SW1E 6LH. (Telephone: 01-828 5676).

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(1898)E

MRC REPRODUCTIVE BIOLOGY UNIT, EDINBURGH

POST-DOCTORAL TRAINING FELLOWSHIP PROTEIN BIOCHEMIST

An active research team studying clinical and biological aspects of infertility and contraception offer training in gamete biology to a BIOCHEMIST with experience in the purification and characterisation of glycopeptides/glycolipids and an interest in gene technology/immunology. The research programme is designed to identify gamete specific cell surface antigens that may be used as the basis for a contraceptive vaccine. The appointment would be for three years at a salary within the range of £7,190 — £12,545 per annum.

Candidates should hold a PhD/DPhil degree or, in certain circumstances, an MSc plus at least three additional years postgraduate experience, and must meet the UK residence requirements set out in the MRC Training Fellowship regulations.

Regulations, application forms and additional information from Miss E. McKay, Administrative Officer, MRC Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW.

Closing date for applications — 31st May 1984. (1929)E

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Pfizer Awards

In October, 1982 Pfizer Central Research at Sandwich, Kent announced that they would make up to six new Awards annually of £1,500 each to support young scientists who had carried out meritorious research at British Universities. The research should have potential application in the search for new human or animal health drugs.

The awards are made by a panel of Pfizer scientists in consultation with senior academic advisers, on the basis of work published in the two years prior to the award. The awards are NOT open to application. Each award may be renewed at the panel's discretion for a second year. The awards are for the personal use of the recipients to further their research.

The 1983 awards were announced in May 1983 and the recipients were:

Chemistry

Dr. (now Prof.) S. V. Ley, Department of Chemistry, Imperial College of Science and Technology, London.

"For his innovative synthetic work exemplified by his approach to the ionohore antibiotic X-1457A and his short synthesis of hirsutene".

Dr. P. J. Kocienski, Department of Chemistry, University of Leeds.

"For the originality of his synthesis of pederal dibenzoate and diumycinol".

Biology

Dr. J. C. McGrath, Institute of Physiology, University of Glasgow.

"For his contribution to the understanding of adrenergic and non-adrenergic transmission".

Dr. B. G. Spratt, School of Biological Sciences, University of Sussex.

"For his work on the role of the penicillin-binding proteins in growth and morphological form of bacteria".

Animal Health

Dr. D. B. Satelle, Agricultural Research Council, Unit of Insect Neurophysiology and Pharmacology, Department of Zoology, University of Cambridge.

"For his work on the pharmacology of insect receptors".

Pharmaceutical Sciences

Prof. I. W. Kellaway, Welsh School of Pharmacy, UWIST, Cardiff.

"For his work on collodial drug-delivery systems including liposomes, nano-particles and the release of drugs from semi-solid dosage forms".

Pfizer will announce the next awards later this month.

Pfizer is a worldwide, research-based company with inter-related businesses in health care, agricultural, speciality chemicals, materials science and consumer products. The Company has a major commitment at Sandwich where it employs approximately 1,500 people, over 500 of whom are engaged in new product research. (1899)N

COURSES continued

EMBO ADVANCED LECTURE COURSE (1984/ 9)

'Molecular Genetics of Mammalian Development

PAVIA, ITALY, 3-15th SEPTEMBER 1984

The Course is intended for 60 research workers or students who wish to start in the area of the Molecular Genetics of Mammalian Development. It will cover both basic concepts and the most recent findings in the field. The format of the Course will be formal lectures, seminars, discussion groups and journal clubs on a wide range of topics. The students will be provided with a book of relevant reprints. Both students and teachers will stay together at Collegio Ghislieri in the mediaeval centre of the city.

The following areas will be covered:

General topics: recombinant DNA techniques in development, germ cells, early development, dedifferentiation and its relationship to embryogenesis, developmental and temporal regulation of genes, tissue specific expression, somatic cell genetics as a tool, hormonal regulation of expression, promotors, enhancers and other regulatory elements, injection of foreign DNA into embryos and its expression, approaches in other animals.

Specific systems include: H-2, HLA and disease, antibody genes, muscle differentiation, nervous system, lens cell, X-inactivation, sex determination, the T/t locus, oncogenes and the cancer problem.

Lecturers will include: C. Morandi, V. Sgaramella (Pavia), A. Carbonara (Torino), G. Tocchini-Valentini (Roma), G. Bulfield, R. Clayton, N. Hastie (Edinburgh), A. McLaren, P. Goodfellow, M. Fried (London), M. Lyon (Harwell), P. Démant (Amsterdam), V. Pirrotta (Heidelberg), V. Chapman (Buffalo), K. Paigen (Berkeley), P. Brûlet, B. Robert (Paris), W.J. Rutter (San Francisco), O. Hägenbuchle (Lausanne).

The Course is organised for EMBO by G. Astaldi Ricotti, C. Morandi, G. Della Valle (CNR Istituto di Genetica Biochimica ed Evoluzionistica and Dipartmento di Genetica e Microbiologia, Universita di Pavia) and G. Bulfield (Edinburgh).

The fee is $280 which includes all meals and accommodation. To register, send off brief CV/ research interests (one side A4) and $50 deposit by *15th June*. Places will be allocated on 'first-come' basis) to:— Dr Grahame Bulfield (EMBO Course 1984/ 9), AFRC Poultry Research Centre, Roslin, Midlothian EH25 9PS, U.K. (Tel. 031-440 2726). (W956)C

UNIVERSITY OF LEEDS MSc IN STEROID ENDOCRINOLOGY

A course of advanced study covering one calendar year will be held in the Division of Steroid Endocrinology from October 1984 to September 1985. Applications should be made before 31st July 1984 to the Secretary at 26-28 Hyde Terrace, Leeds LS2 9LN (Tel: (0532) 454436) from whom application forms and brochure may be obtained. (1934)C

ORGANIZATION OF THE EUKARYOTIC GENOME
MOBILE ELEMENTS IN THE EUKARYOTIC GENOME
(8-12 October 1984, Montpellier, France)

A FEBS Lecture Course on the *Organization of the Eukaryotic Genome* will take place near Montpellier, France, between October 8-10. The Course will be followed by a Symposium on *Mobile Elements in the Eukaryotic Genome* (October 11-12) organized by the IUB-IUPAB Interest Group "Genome Structure and Expression".

Invited speakers include:

C. Bostock (Edinburgh), J.C. Bregliano (Clermont-Ferrand), P. Di Nocera (Naples), J. Doly (Villejuif), G. Dover (Cambridge), H. Feldmann (Münich), J. Filipski (Paris), D. Flavell (Cambridge), G. Georgiev (Moscow), G. Grimaldi (Naples), N. Hastie (Edinburgh), I. Horak (Würzburg), W. Jelinek (New York), E. Kuff (Bethesda), L. Manuelidis (New Haven), M. Meunier-Rotival (Paris), P.W.J. Rigby (London), S. Roeder (New Haven), J.H. Rogers (Cambridge), F. Rougeon (Paris), C. Schmid (Davis), J. Szoztack (Boston), B.K. Tye (Ithaca), E. Ullu (New Haven).

Attendance to both events will be limited to 120 people. Those interested in participating should contact either the Organizer of the FEBS Course, Dr. G. Roizès (Laboratoire Structure, Fonction et Evolution du Génome Eucaryote, Institut de Biologie, Boulevard Henri IV, 34000 Montpellier, France) or one of the Organizers of the Symposium, Drs. M. Singer (N.I.H., National Cancer Institute, Bethesda, Md. 20205, U.S.A.) and G. Bernardi (Laboratoire de Génétique Moléculaire, Institut Jacques Monod, 2 Place Jussieu, 75005 Paris, France). (W950)C

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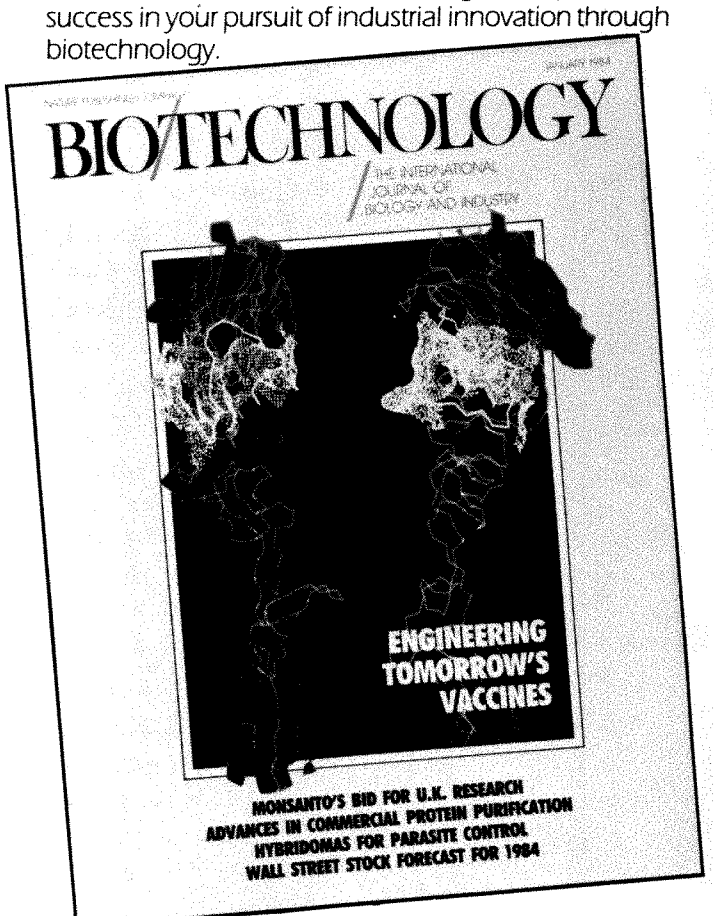
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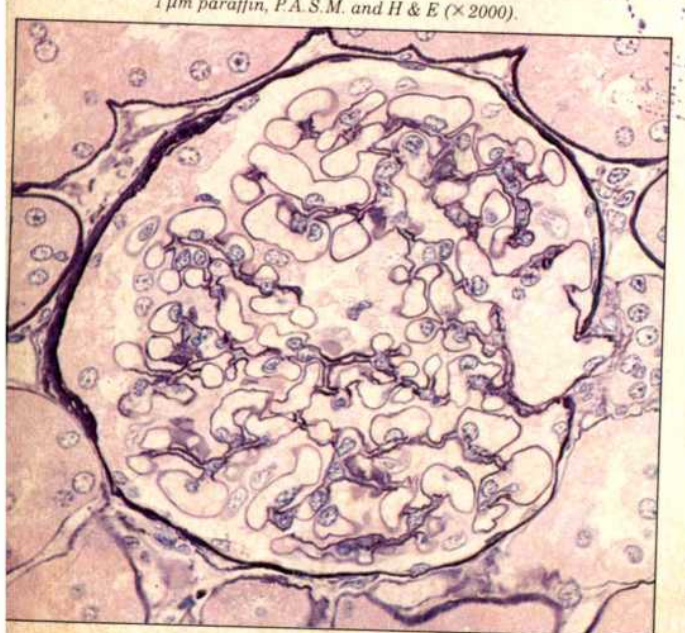
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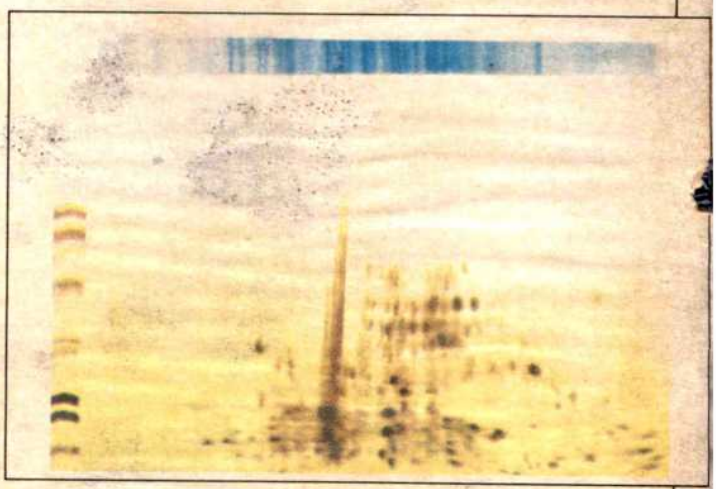
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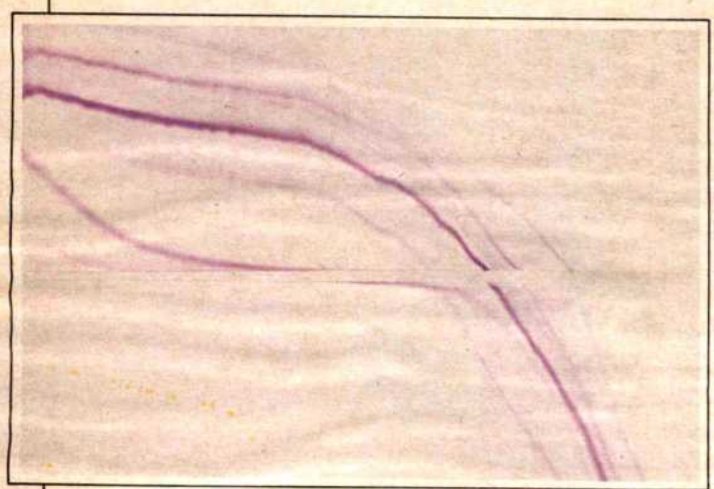
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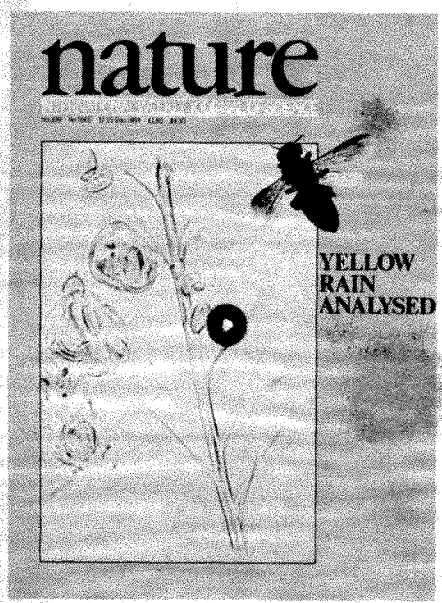
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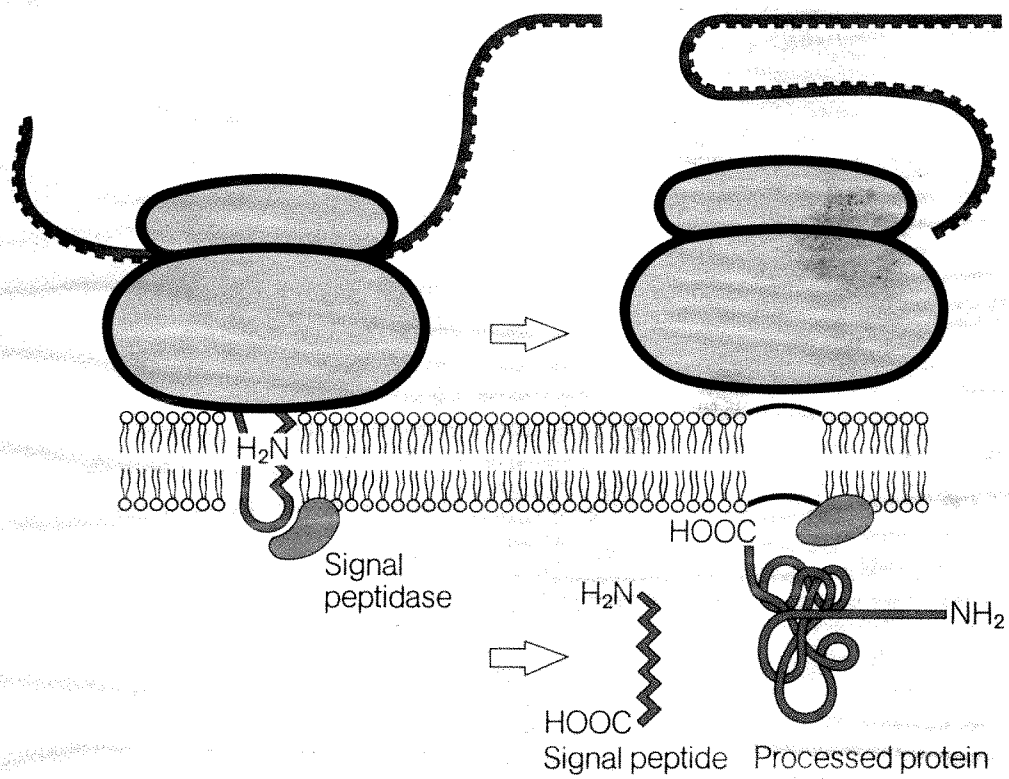
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DAY 1 - ONCOGENES,
DAY 2 - THE CANCER CELL,
DAY 3 - PROSPECTS/PROBLEMS.

MONDAY, 10 September
Exhibition Viewing: 8:30am — 6:30pm
Recruitment Center: 9:30am — 6:00pm

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ONCOGENES AND GROWTH FACTORS — WHAT THEY ARE AND WHAT THEY DO

9:00 — 9:15	**Chairman**
9:15 — 10:00	Oncogenes *R. Weinberg (MIT)*
10:00 — 10:45	Growth factors *M. Spom (NIH)*
11.15 — 12.00	HTLV *R. Gallo (NIH)*
12.00 — 12.30	Clinician's view *S. Broder (NIH)*
2.00 — 4.00	Round table with morning speakers
4.30 — 5.30	The cancer cell *J. Cairns (Harvard)*

TUESDAY, 11 September
Exhibition Viewing: 8:30am — 6:30pm
Recruitment Center: 8:30am — 6:00pm

DAY 2

THE CANCER CELL — HEREDITARY CANCER, TUMOR PROMOTERS AND CARCINOGENESIS

9.00 — 9.15	**Chairman**
9.15 — 10.00	Gene regulation *P. Leder (Harvard)*
10.00 — 10.45	Hereditary cancer *R. White (Utah)*
11.15 — 12.00	Carcinogens and promoters *I.B. Weinstein (Columbia)*
12.00 — 12.30	Clinician's view *F. Gilbert (Mt Sinai)*
2.00 — 4.00	Round table with morning speakers
4.30 — 5.30	NCI program

WEDNESDAY, 12 September
Exhibition Viewing: 8:30am — 3:00pm
Recruitment Center: 8:30am — 3:00pm

DAY 3

PROSPECTS AND PROBLEMS — DRUG RESISTANCE, MONOCLONAL ANTIBODIES AND NEW DRUGS

9.00 — 9.15	**Chairman**
9.15 — 10.00	Diagnostics and therapeutics *G. Todaro (Oncogen)*
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nature

New chance for East-West talks

The Soviet proposals put at Stockholm last week have been too quickly dismissed in the West as an old recipe for arms control. That they may be, but why not talk about them?

THE Soviet decision to pull out of this year's Olympic Games has inevitably overshadowed the Soviet Union's presentation of a string of arms control proposals to the Stockholm conference on European security, which reconvened on 8 May. The first development, ostensibly on the grounds that Soviet athletes would be physically endangered in Los Angeles, is regrettable at such short notice but may in the end be an important contribution to international understanding; the Olympic Games have been, for far too long, occasions for unbridled displays of chauvinism. And on the face of things, the Soviet proposals at Stockholm are both familiar and unacceptable for reasons which by now should be widely understood. Yet that should not prevent the other participants at Stockholm, one of the meetings called within the framework of the Helsinki agreements, from taking this development as an opportunity for revivifying the East–West dialogue on arms control, distinctly in abeyance since the collapse of the nuclear negotiations at Geneva at the end of 1983.

The new Soviet package is a mixed bag of the familiar and unfamiliar. Predictably, it includes (at the head of the list) the demand that nuclear powers should declare that they will not be the first to use nuclear weapons. This old proposal is presumably included for form's sake, since the Soviet Union is well aware that for the past quarter of a century, the defence of Western Europe has been based on the assumption that battlefield nuclear weapons might be necessary to counter a successful conventional incursion from the East. But a no-first-use declaration would also have the contradictory effects of helping to encourage conventional conflicts that could escalate into nuclear wars and of encouraging nuclear powers to keep their nuclear arsenals intact (because of the risks inherent in taking such a declaration at its face value). At Stockholm, however, the Soviet Union has gone further by advocating a treaty under which all European powers would agree not to be the first to use either conventional or nuclear forces. At first sight, this is simply an attempt to revive the old idea of a non-aggression pact between East and West in Europe, a notion which pragmatic Western governments have consistently resisted on the grounds that such an agreement could be only a cosmetic device for pretending that political and ideological differences are less serious than they seem. But in the absence of constructive talks about serious arms control, it would be constructive to know precisely how else the Soviet Union proposes that conflicts should be settled. A standing commission for the discussion of differences of the kind from which European wars could spring would be no bad thing.

Chemical weapons

Freezing (and then reducing) military budgets, another of the Soviet proposals, is by contrast meaningless, pointless and probably unacceptable even in Moscow. Just how powers with international commitments, such as the Soviet Union and the United States, would limit the resources devoted to preparations just for European wars is anybody's guess. Eliminating chemical weapons from the European theatre is, however, a suggestion that deserves serious consideration. None of the governments with interests in Europe can be cheerful about the present need to plan for the use of chemical weapons, and while the chief among them would probably be able deliberately to violate such an agreement (or to counter a violation) within weeks if not days, the risks in being caught napping would not be overwhelming. So there is a case for talking constructively about the central problem in any such proposals — what arrangements could be made for verifying compliance with such an agreement? Technically, as the accusations and counter-accusations of the war between Iran and Iraq have shown, this is an exceedingly difficult issue — which is a good reason why it should be taken up in good time.

The remainder of the Soviet package is still more interesting at least in the sense that discussion can only be fruitful. First, there is a suggestion that the present arrangement under the Helsinki agreements whereby military exercises must be notified in advance should be extended to all movements of troops and military equipment above a scale yet to be fixed. Although intelligence-gathering by both sides in Europe is probably now sufficient for each side accurately to know what the other is about, such an agreement would be well worth having as a demonstration of openness and truthfulness. The West should jump at it.

Zones

The proposal that some consideration should be given to nuclear-free zones in Europe — what many in the West consider to be the most obviously-poisoned chalice in the new Soviet package — also deserves serious consideration, the obvious dangers notwithstanding. Again, more than a quarter of a century has passed since the Polish foreign minister Rapacki proposed that there should be a nuclear-free zone in central Europe (including both Germanies and Poland) agreed only among the governments directly affected. More recently, there has been a great deal of enthusiastic talk in Scandinavia about the potential value of a nuclear-free zone stretching from Finland in the east to Denmark in the west. The Soviet proposal to the Stockholm meeting last week raises the further question of a nuclear-free zone covering the "Balkan peninsula" without specifying whether that term embraces Turkey. All such schemes have the disadvantage that their benefits are much less real than they seem. But although it might lead to nothing, the West should again jump at the chance of such a discussion, if only for its educative value (for both sides).

Of the three proposed nuclear-free zones, that in central Europe is the more important — and more difficult. One almost preliminary difficulty is that of knowing what is meant by the term "nuclear-free". In central Europe, the only possible meaningful interpretation must include the interdiction of the physical presence of nuclear explosives, by whichever organization they are manufactured. (The governments concerned do not manufacture nuclear weapons on their own, but provide house-room for other people's.) Since, in central Europe, it must supposed that nuclear-free territory would quickly reacquire nuclear weapons if hostilities broke out, arguments that apply elsewhere about the propriety of command and control installations are academic. On the other hand, there are serious practical difficulties about the boundaries that might be drawn for a nuclear-free zone, given that an agreement including the whole of West Germany would effectively exclude NATO nuclear weapons from central Europe. Yet both sides would learn a great deal from a discussion of the problems. Why, "without prejudice" as the lawyers say, do they not agree to talk about them? □

Star wars unworkable

President Reagan's best friends should tell him that his star-wars scenario is an illusion.

PRESIDENT Ronald Reagan's plan for a system of "star wars" defences against nuclear attack will not work. The scientific community, which the president asked a little over a year ago to invent the means of rendering nuclear weapons "impotent and obsolete", knows that it will not work, and has said so. The president's advisers, including his science adviser, Dr George Keyworth, know it too, but are afraid to say so. Instead, they have invented a different and more plausible version of what Mr Reagan wants in the hope that nobody will notice the difference. They will of course be found out; the danger is that they might get away with it for long enough to do substantial mischief to the cause of arms control.

Revisionism

The revisionist version of the star wars idea goes something like this: yes, it does seem rather difficult to think of a way to defend the US people against a nuclear attack, but there is no need to look for a near-perfect defence right away. As a first step, the United States might construct a star wars system that could shoot down a fair proportion of Soviet intercontinental ballistic missiles (ICBMs) launched against the United States in a nuclear war. Thus the degree of confidence with which the Soviet Union could launch a preemptive attack on US missiles would diminish; since they would have reason to fear American retaliation, deterrence would be strengthened. Later on, perhaps, the defensive system might enfeeble strategic nuclear weapons so much that they would become too unreliable to be used.

What is wrong with this argument? Plenty, as it happens. But the first thing to say is that it bears virtually no relation to what President Reagan actually said in his star wars speech 14 months ago. In that speech, the president did not propose a defensive system as a means of preserving the ability of the United States to retaliate against a Soviet preemptive strike, but as a way to make retaliation unnecessary. Here is what he said.

> Up until now we have increasingly based our strategy of deterrence upon the threat of retaliation. But what if free people could live secure in the knowledge that their security did not rest upon the threat of instant US retaliation to a Soviet attack; that we could intercept and destroy strategic ballistic missiles before they reached our own soil or that of our allies?

There is nothing ambivalent about that; what the president proposed was a shield through which so few nuclear missiles could leak that the United States would not even have to threaten the Soviet Union with retaliation in the event of a nuclear attack. Is it feasible? Almost certainly not, for reasons that have been fairly and lucidly explained in a new report from the Office of Technology Assessment (OTA). The OTA study is especially significant because it was prepared with the benefit of full access to classified data by a body which is traditionally careful to avoid partisan stances. Even so, it found the prospect of a near-perfect defence "so remote that it should not serve as the basis of public expectation or national policy".

Pessimism

The reasons for pessimism are clear. At least part of a successful defensive array would have to be based in space, where it would itself be hopelessly vulnerable; even if it could somehow be protected, the defensive system would have to shoot at so many boosters, warheads and decoys that its data-processing requirements would be far beyond what is presently imaginable; none of the weapons yet proposed are based on mature technology, whereas countermeasures are; to destroy Soviet missiles before they could deploy their multiple warheads would require such an instant response that a decision to activate the system might have to be taken by machines rather than people.

The biggest flaw, however, is that the principal characteristic of nuclear weapons — the ability of tiny objects to cause massive destruction — continues to make a nonsense of the notion of a "near-perfect" defence. There are, and there always have been, some respectable arguments for defence. After all, the difference between an attack in which 250 megatonnes were detonated over the United States and an attack in which only 5 megatonnes (about one two-thousandth of the Soviet arsenal) got through cannot be ignored. While the former could kill half the population and injure most of the rest, the latter, if cities were preferentially targeted, could kill several million people and injure 10 million more. The scores of millions saved by the successful interception of the 245 megatonnes would have reason to be grateful, but could hardly regard the destruction of the most great cities and the death of millions of their fellows as a near-perfect defence. The temptation to retaliate — and the need to deter — would remain.

So the president's vision of a future in which a leakproof defence makes nuclear weapons obsolete and the threat of retaliation unnecessary is a vision that cannot be given technical expression. The taxpayers of the United States employ people whose job it is to keep the president informed about the limits of technology. Dr Keyworth is one of them. Sadly, there is no evidence that he is willing to give Mr Reagan the bad news; indeed, there are disquieting signs of a witch hunt against those scientists who have spoken out honestly. Some have been accused of defeatism, others of disloyalty. It is, however, an entirely misguided sense of loyalty that has persuaded the revisionists to fudge the issue by arguing that if "star wars" cannot defend everything they can at least defend our ability to retaliate. That is a travesty of the promise President Reagan held out in his speech on 23 March, and an argument that will do him no good politically. Neither Congress nor the general public will be pleased to discover that the panacea in which they have been asked to invest billions of dollars is intended as the strategic equivalent of a hardened silo.

Dishonesty

That is why the revisionist argument is dishonest. But is it wise? There is in general something to be said, as Freeman Dyson argues in his recent book, *Weapons and Hope* (Harper & Row, New York and London), for the moral superiority of defensive weapons. Here the arguments for star wars and for civil defence converge; it is worth trying to save the lives of some even if it is not possible to save the lives of all. The snag is that no policy or action in the domain of nuclear weapons takes place in a vacuum; each increases or diminishes the likelihood of a war being fought with nuclear weapons. There is unfortunately little reason to believe that possession of star wars defences will diminish the likelihood of war, and some reason to believe it will increase it.

That is because neither side, faced with the development of a defensive shield by the other, will acquiesce in the president's aim of making nuclear weapons "impotent and obsolete". Instead, by deploying a defensive shield of impressive but imperfect capabilities, each side gives the other an extra reason to strike first; the shield will be less effective against an unexpected massive first strike than it will against a ragged retaliation by the survivors of an attack. Nor will the shield necessarily save lives. A superpower confronted with a star wars defence could decide to aim a higher proportion of its missiles at cities to preserve its ability to deter. Finally, the development of a star wars system makes the chances of reducing the number of nuclear weapons increasingly remote; if your enemy has a shield you need more arrows.

It is argued by some that even if the defensive notion turns out to be neither feasible nor desirable, there is no harm now in pursuing the research that will keep the United States' options open in the future. The harm, of course, is in allowing the quest for rational progress on arms control to be delayed by the search for technical panaceas; in believing that the next weapon or gadget you invent will make its predecessors redundant. *Vide* Dr Keyworth testifying on star wars research to the Senate Foreign Relations Committee last month: "While I see absolutely no need to violate any provision of existing treaties while we investigate these options, I am very leery of entangling ourselves in future treaties until we know more". □

British Technology Group

Commons committee shows no mercy

A FIERCE attack on the British Technology Group (BTG) was launched this week by the House of Commons Public Accounts Committee (PAC). The chief criticism centres on the investment by the National Enterprise Board (with the National Research Development Corporation one of the two components of BTG) in the company called NEXOS, set up in January 1979 to develop and sell an integrated office automation system. PAC complains that it is "far from satisfied" with the explanation of how the company lost £31 million before it went into receivership in October 1981, and asks that the Department of Trade and Industry should carry out an investigation which "we shall wish to examine".

PAC says it has been hampered in its investigation of the collapse of NEXOS Office Systems Ltd by the fact that the Auditor and Comptroller-General does not have access to the accounts of the National Enterprise Board (NEB). It has, however, been able to establish to its own satisfaction from evidence given by officials that NEXOS, intended to market an office automation system based on word processors, facsimile machines and voice systems, failed because of over-optimistic projections of its sales, "unsatisfactory" relations with component suppliers and "management deficiences".

The PAC report explains that NEXOS when founded was dependent on a commercial agreement with Logica VTS to be the sole source of supply of a word processor which had not been developed when the company first began trading and which was eventually put into production after the date first promised. PAC says that NEXOS "was obliged to purchase, effectively under a cost-plus contract", a word processor over whose development and quality it had no control, and under a contract that did not specify what should happen if "something went wrong".

PAC says that the trading loss reported for the period of NEXOS's existence of £17 million seems "extraordinarily high" by the yardstick of the company's best sales of £8.9 million in 1981, the year in which it collapsed. The committee also asks "whether any consideration was sought in respect of the £5.2 million loss" quoted for research and development and asks why £0.6 million of public money was written off under the heading of "bad debts".

Apart from its particular interest in this investment, PAC emphasizes that the NEXOS collapse is another reason why BTG should be made more directly accountable to the British Parliament. The report is likely to play an important part in the British Government's legislation for a formal merger of NEB and NRDC, which has been planned for the past two years. Evidence given to PAC confirms that the unified organization is likely to function more as a sponsor of innovation along the lines of NRDC than as a source of public money to be used as venture capital, the chief role of NEB in recent years.

PAC is also, however, critical of BTG's handling of INMOS, the company set up in 1976 to manufacture silicon chips in the United Kingdom. Acknowledging that the venture was a "high-risk" undertaking, PAC says it is disappointed that sales forecasts have consistently been unfulfilled but seems even more dismayed that the company's plan to employ 4,000 people by the mid-1980s has not yet been achieved, and that employment at Colorado Springs in the United States is still greater than that in Britain.

The future of BTG will no doubt be clouded by these opinions. The formal merger of the two component organizations apparently waits on parliamentary time (and the government's stomach for the argument it will provoke), but by all accounts BTG has now submitted its corporate plan to the Department of Trade and Industry, on which basis it is likely that the government will outline for BTG the manner in which it will be allowed to continue. One matter still unresolved is the promise, made at the Prime Minister's seminar on technology last September, that NRDC will relinquish its first refusal of innovations arising in the public sector. □

British universities

Tenure principle to be trimmed

THE British Government seems to have hit on the one device for the abolition of tenure in British universities that is likely to satisfy British academics. The Secretary of State for Education and Science, Sir Keith Joseph, announced last week plans to set up a "Statutory Commission" whose function will be to amend university charters to remove from academic contracts of employment explicit references to the expectation that academic appointments will normally continue until "normal retirement age".

The first reactions of university vice-chancellors to the proposal are generally speaking those of people fearful of being about to be hanged who have had their sentences commuted to life imprisonment. For the past four years, the British Government has made no secret of its distaste for the principle of tenure, and two years have passed since the Committee of Vice-Chancellors and Principals put forward proposals for amending the standard conditions of tenure. In particular, the committee suggested that probationary periods for new academics should be lengthened, and raised the possibility of periodic reviews of individuals' performance.

In a letter to the committee last week, Sir Keith Joseph complained that "with one or two limited exceptions, there has been no response" and said that the problem is probably one that the universities cannot "resolve by themselves". His letter also, however, makes plain that the government's objective is that only new contracts should provide for "reasons of redundancy or financial exigency".

The vice-chancellors, who saw the government's letter for the first time at their meeting last Friday, are relieved because the threat of blanket legislation explicitly governing the conditions of academic employment has thus been withdrawn. Even so, the government will have to obtain an Act of Parliament delegating power to legislate to commissioners, whose task will be to rewrite university charters in line with the terms of reference eventually embodied in the act.

Meanwhile, the new development will provide academic lawyers with a rare opportunity for archival work. The proposed statutory commission will be the first since that which, in the early 1920s, arranged to bring the universites of Oxford and Cambridge within the umbrella of public support by giving the central administration of the two universities the right to receive funds from the then new University Grants Commission. Two earlier commissions (in the 1850s and 1870s) had similarly been concerned with the affairs of Oxford and Cambridge.

The enforced reduction of academic staffs in British universities in the past three years appears, so far, to have been accomplished without the principle of tenure being challenged directly. Part of the explanation appears to have been the relatively generous conditions on which it has been possible for academics over the age of 50 to retire early, but it seems clear that the academics' representative organization, the Association of University Teachers, has been anxious to avoid confrontation for fear of having to hazard the principle of tenure.

The universities seem also to have won a victory in their war of attrition with the government by securing an undertaking that a proposed investigation of the efficiency of academic administration, to be conducted at six universities not yet chosen, will not investigate the conduct of academic affairs. This inquiry is likely to be completed in two years. □

Japanese computers

Fifth-generation machines here

Tokyo

JAPAN'S fifth-generation computer project has taken a big step towards its preliminary goals with the successful construction of a prototype relational database machine — the first of its kind.

The fifth-generation computer project aims at the development, by the 1990s, of a completely new form of computer that can communicate in everyday language, "reason intelligently" and bring enormous amounts of stored knowledge to bear on any problem that the user might set it. The machines are intended to be cheap and reliable enough to be used in homes and offices. Some talk as if they will revolutionize society by making freely available a huge fund of expertise that will vastly "amplify human intelligence".

When the project was announced by the Ministry of International Trade and Industry (MITI) in 1981, its sheer boldness and originality astonished people in the West. Japan, considered weak in fundamentally new ideas, seemed overnight to have produced a plan that would give it an unassailable lead in advanced computer technology. One expert even uncharitably described the plan as a "technological *Mein Kampf* ".

Since then, Western governments have responded more rationally with new projects of their own. There are, for example, advanced information technology projects in the United Kingdom, France and West Germany, together with the European Communities' Esprit programme, while the schemes of the United States' Defense Advanced Research Projects Agency were directly inspired by Japan.

The fifth-generation computer will be composed of three main functions: an intelligent interface allowing it to communicate flexibly with humans by speaking, writing or drawing; problem-solving and inference functions allowing problems to be solved by deductive and inductive inference as well as by making sensible guesses where knowledge is incomplete; and a knowledge base function able systematically to store and retrieve not only vast quantities of data but also judgements and test results.

It is within this third function that 45 researchers at the Institute for New Generation Computer Technology (ICOT), set up by MITI to run the project in cooperation with Japan's big eight electronic manufacturers, have now announced a major step forward. Success with the construction of the relational database machine follows by five months the completion of the hardware of one of the other two essential parts of a prototype fifth-generation machine — a sequential inference machine.

The novelty in ICOT's machine is that relational software has been replaced by specially developed hardware. Not all the control software is complete so the machine is not actually doing anything yet. But when in operation, it will be the first true relational database "machine", working two orders of magnitude faster than anything else available. Named Delta, the device consists of two parts: a relational processor complex containing pipelined relational algebra engines (built by Toshiba) and a hierarchical memory subsystem (built by Hitachi), plus an interface (built by Oki). The aim is to be able to connect it with the prototype sequential inference machine by next spring. ICOT hopes then to have two-thirds of a prototype fifth-generation machine, providing the programming environment in which to tackle the really big problems ahead.

Connecting the two together requires that both operate on the chosen "kernel" language of extended PROLOG, the advanced logic programming language invented in France and developed in the United Kingdom. There are several tricky software problems to be solved.

That both ICOT's sequential inference machine, which broke new ground as hardware by allowing extended PROLOG to be used as its machine language (see below), and the relational database centre on relations between things is no accident. ICOT's visionary director, Kazuhiro Fuchi, sees the predicate logic they use, established by Godel in the 1930s, as the key to building knowledge information processing systems. Indeed, he has said that the present generation of computers, based on the Turing theory published in 1936, may one day be seen as a "gigantic historical detour".

The fifth-generation computer project is scheduled to last ten years. The first three years, up to next spring, seem likely to achieve the goal of a prototype machine to be used as a base for further research. Eventually, however, machines will have to work at incomparably greater speeds than at present. The goal is 100 to 1,000 million logical inferences per second (Lips), compared with just 30,000 Lips for the present machine.

This speed can be achieved only by switching to some sort of parallel architecture for the sequential inference machine, probably based on a data flow model (in which the University of Manchester is an acknowledged leader). The project also has to solve the stupendous problems of developing syntactical analysis systems allowing communication with humans in natural language, making a complete revision of PROLOG for parallel use, developing knowledge-base machines utilizing parallel operations and pattern information processing systems.

What has been achieved so far consists of the rapid and determined development of ideas well researched elsewhere. The four-year intermediate stage of the project beginning in 1985, however, requires radical innovations — on time. Those years will decide whether a Japanese fifth-generation computer will emerge in the 1990s or whether it will have to be postponed until the world of information technology is much further advanced.

This year's budget for the project is Y5,100 million (£16 million). Later budgets, it is said, will depend upon "progress in research". **Alun Anderson**

Logic and language for the new generation

RELATIONAL databases are not in themselves new — their mathematical basis was laid out in 1972. What makes them distinct from more conventional data bases, and particularly suits them to "intelligent" applications, is their simplicity of organization. Entries consist purely of relations (essentially two-dimensional tables).

This provides access to any datum in the base either directly or through association and allows data sets to be rearranged in new combinations. If, for example, a set of statistics about university students and their examination results is stored in a relational data base, it is easy to ask the base to provide, say, the names of all students who scored exactly 85 per cent in their examinations. Traditionally, this would be accomplished only by searching through the whole data base. The facility to retrieve data flexibly (by relational algebra) has obvious advantages for intelligent operations, for it will never be clear in advance just how knowledge will be used.

PROLOG, the chosen kernel language, was originally devised for solving problems that involve symbolic representations of objects and relationships between them. To the average programmer, accustomed to writing sequential instructions, PROLOG is something of a surprise.

Thus programmes are written in terms of facts, for example:

```
Tim likes doughnuts
```

written as

```
likes (Tim, doughnuts)
```

rules, for example:

```
Tim likes any object
provided it is sweet
```

and questions, for example:

```
Does Tim like doughnuts?
```

written as:

```
? likes (Tim, doughnuts)
```

which will cause a search to be made to see if a precisely matching declaration can be found in the data. Building from these very simple roots, extremely complex logical arguments can be advanced and complicated inferences be made from one statement to another. Alun Anderson

Nuclear test fallout: 1

US Government arraigned

Washington

A FEDERAL judge in Utah has ruled that fallout from nuclear bomb tests was the "proximate cause" of certain types of cancers developed by nearby residents, and that the government had failed to take proper precautions. Ten plaintiffs, who had developed thyroid cancer, breast cancer or leukaemia, were awarded a total of $2.7 million in damages. All but one of the ten have since died from their illnesses. The judge ruled that another 14 plaintiffs, who developed other forms of cancer, had not proved a link to the fallout.

Although the decision is certain to be appealed by the government, it has given encouragement to thousands of others who are claiming injury from exposure to low-level radiation generated by the government's nuclear programme. These include 375 other residents of southwestern Utah whose cases have not yet come to trail; uranium miners, who say they were not advised of the risks of radiation exposure; workers at the Nevada Test Site; sheep ranchers in Utah who claim their flocks were killed by fallout; and "atomic veterans" who served at Hiroshima and Nagasaki in Japan and at nuclear test sites.

The judge's decision rested heavily on a finding of negligence on the government's part in failing to carry out a proper monitoring programme that could have measured radiation levels in nearby areas and in failing to warn residents to recommend simple precautions. The plaintiffs lived approximately 100 miles from the Nevada Test Site during the 1950s and early 1960s when above-ground nuclear testing was carried out. The plaintiffs were able to sue under a special law that allows findings of negligence against the government in certain cases.

In establishing the nuclear tests as the most likely cause of the cancers, the judge accepted the conclusions of several controversial studies that found higher than normal cancer rates in the region. A 1979 study by Dr Joseph Lyon of the University of Utah, cited in the ruling, found nearly twice the incidence of leukaemia in children exposed to the fallout as compared with children who were born long enough before testing began to escape exposure. That study, however, has been strongly criticized, most recently by Dr Charles Land of the National Cancer Institute; Land argues that the rate of the exposed group is actually no higher than the normal rate of Utah, and suggests that the lower rate of the earlier group may be the result of under-reporting of the disease at a time when that remote part of Utah had relatively poor health services.

The three cancers that the judge said were linked to the nuclear tests are the most sensitive to radiation induction. The plaintiffs received radiation doses of, typically, a few rads. According to several independent experts, these doses — particularly in the case of the leukaemias — are enough to attribute a significant though not overwhelming probability of causation to the nuclear tests. **Stephen Budiansky**

Government veto for Warsaw rector

THE Polish Ministry of Science, Higher Education and Technology has refused to confirm in office the newly elected rector of Warsaw University, Dr Klemens Szaniawski. This decision runs counter to pledges given through the goverment press spokesman, Mr Jerzy Urban, that university officers elected in accordance with the 1982 Higher Education Act would be confirmed in office "with a possible exception". The exception Urban referred to, however, was Dr Janusz Onyszkiewicz, the mathematical logician recently elected to the Warsaw University senate who had been the chief press spokesman of Solidarity. Dr Szaniawski, however, was not even a member of Solidarity.

The swift response from the ministry on Dr Szaniawski is surprising. According to the procedure laid down in the 1982 act, the minister had two weeks to reach a decision. In the matter of Dr Onyszkiewicz's election to the senate (a post far less significant for the running of the university), a stalemate has been allowed to develop. The ministry notified its objection to Onyszkiewicz's election (on the grounds of electoral "improprieties") at the end of March. The outgoing rector, Dr Kazimierz Dobrewolski, said that he was working on his reply and then said he could not act further because he was unclear whether he should convene the old or new senate.

By vetoing the election of the new rector within three days, the ministry has clearly indicated its commitment to the slogan enunciated by Politburo member Tadeusz Porebski, at the plenary meeting of the new Association of Polish Students. The party and state leadership, he said, is "determined to counteract the capture of management posts [in education] by people who do not guarantee the socialist character of the school". Earlier official warnings had said only that the authorities could not tolerate university elections being used to secure a power base for "opposition" elements.

Dr Szaniawski, however, is an essentially moderate figure who, during the Solidarity era, acted as a mediator between the interests of the party and the demands of Solidarity in the drafting of the new higher education and censorship bills. Vera Rich

Nuclear test fallout: 2

Claim and counter-claim

Canberra

THE federal Australian Labor Government is under growing pressure from its own backbenches and the Labor premier of South Australia, Mr John Bannon, to furnish more information about alleged secret nuclear tests at Maralinga in 1963, the allegedly unmarked burial of radioactive equipment and the storage of British tactical nuclear weapons on Australian soil during the 1958–61 partial test ban. It is also alleged that human guinea pig experiments were conducted during the seven official tests at Maralinga in 1956–57. Former civilian and ex-military workers on the test sites have risked prosecution under the Official Secrets Act to come forward with accounts — some anonymous, some in part mutually corroborative — that suggest unacknowledged nuclear test operations. These include stories of badly burned military personnel "berserk with pain", civilian workers told to "climb ladders" or to remain unwittingly in areas five miles away, during the tests carried out in the 1950s, and of fireball and mushroom-cloud explosions observed in 1961.

These claims follow the death-bed allegations by a former Royal Air Force technician, Mr John Burke, that he had found four aborigines dead in a bomb crater after one of three previously undisclosed tests in 1963. His claim to have informed the Commonwealth police of the deaths has provoked charges of an official cover-up, since no record has yet surfaced. Mr Burke died on 1 May of stomach cancer which he believed could be directly attributed to the tests.

Claims by the UK Ministry of Defence (MoD) that the three 1963 implosion trigger experiments were one-ton chemical blasts involving polonium may explain the aborigine deaths, but not, perhaps, the extent of the damage reported in the 1961 blasts, in which steel constructions are said to have been oxidized and lead bricks fused at a distance of one mile. The MoD "confirmation" that the 1963 tests were extremely minor sits uncomfortably with the recently-leaked unexpurgated version of the Pearce report on residual contamination at Maralinga, in which 20 kilograms of plutonium is reported to have been spread over four Maralinga sites during 1956–63, resulting in plutonium plumes of above-tolerance radioactivity extending over thousands of hectares.

The anecdotal eyewitness reports are at present unsubstantiated, but South Australian aborigine leaders say they are planning international court action and Mr Bannon has written to Mrs Margaret Thatcher, the British Prime Minister, and Mr Neil Kinnock, the British Opposition leader, for full disclosure. **Jeffrey Sellar**

Biochemistry in Japan

Applying fundamentals

Tokyo

INCREASED support for fundamental research in the life sciences is planned in a report to Prime Minister Yasuhiro Nakasone by Japan's top science policy-making body, the Council for Science and Technology.

Concern about the long lead of the United States in basic research in molecular biology and the effect this will have on the commercial exploitation of genetic engineering lies behind the report, and swift action is expected on its main recommendations — increasing the numbers and mobility of young researchers and strengthening the technical back-up for biotechnology. But university researchers are likely to be disappointed, for although the report is entirely concerned with "fundamental" research, the government sees this as "essential research fundamental to planned applications". Expansion of truly fundamental research in the universities is unlikely.

To prepare the report, the council, which includes the heads of the science and finance-related ministries and agencies as well as industrial leaders, took special advice from both a life sciences group drawn from government, universities and industry and from a special committee comprising 22 of Japan's leading molecular biologists. Nakasone is expected to accept the report within a few weeks, when the ministries will be expected to work out how it might best be implemented. The financial implications should be included in ministry draft budgets by the end of the year.

Professor Hiuga Saito, head of the Tokyo University's Applied Microbiology Institute, who was the chairman of the council's special committee, stresses that the most important point made by the report is the need to give more young people a training in biotechnology, perhaps by means of special fellowships which would allow young scientists to carry out original research — a possibility already under consideration by the Ministry of Education, Culture and Science (MESC).

The report particularly emphasizes that all three of Japan's research groupings — the universities, government research laboratories and industry — must be encouraged to work together more closely. The idea of a special research institute, supported jointly by government and business, where young people could be trained, is floated. But the university side shows little enthusiasm for that project, and the chances are that it will proceed no further.

A more promising proposal is the removal of restrictions that hamper original research. Increased flexibility is, for example, to be encouraged in the research system so that research proposals not fitting into the conventional "project" funding can find support. Increased freedom for young people to carry out individual research, and increased flexibility in the supply of equipment, perhaps by arranging for the rental of expensive equipment, are other possibilities.

University workers may gain from the recognition in the report that the pace at which biotechnology is advancing means that scientists can stay on top only if they can circulate around top laboratories — increased exchange of researchers is called for, both within Japan and between Japan and foreign countries, including more visits from foreign scientists.

The report also calls for the government to step up efforts to secure the basic resources of biotechnology. Calls are made for the creation of a larger collection of microorganisms, including those with special characteristics and from unique environments, as well as increasing stocks of cell lines, particularly those useful for the creation of hybridomas.

More rapid and thorough dissemination of data, particularly DNA and protein sequences but also data on the special characteristics of organisms and their life cycles, is seen to be important. A new computerized database to complement those existing elsewhere may result. Finally the report argues for the provision of more sophisticated tools for molecular biology — automatic DNA and protein synthesizers and analysers, and microinjection and laser techniques.

If all these measures are put into effect, the result should be a major fillip for research in industry but not for the universities. What the universities lack at present is not money for molecular biology but permanent research staff. An increase in staff (or the new college or department of life sciences that Professor Saito would like to see) is, however, almost impossible under the present administrative system because the government has a long-term commitment to reduce the number of civil servants — and university researchers are civil servants, employed by MESC.

Industry can expect to benefit quickly from an increased flow of researchers and resources. Indeed, confidence that industry can do its own biotechnology research and apply that research with superb efficiency is expressed in a report from a private advisory body, the Japan Economic Research Centre, published after the government council's report. The report predicts that Japan will be the world leader in biotechnology by the end of the century: joint ventures and technical links with foreign companies will bridge the gap over the next three to five years and then Japan's proven ability to commercialize technology should enable it to begin to move into the lead. The only note of caution behind such predictions is the proviso that the universities will have to provide a good supply of trained personnel in search of jobs.

Alun Anderson

Molecular sequences

UK community seeks access

BRITISH molecular biologists, frustrated by inadequate computing facilities, are attempting to persuade research councils and cancer charities to finance a new national computer link-up and software support scheme for protein and DNA sequencing. Their problems will be aired at a special meeting next month of the Inter-Research Council Coordinating Committee on Biotechnology with interested parties.

The stimulus for the meeting was a letter from prominent researchers addressed to the three research councils that support molecular biology and to the Imperial Cancer Research Fund and the Cancer Research Campaign. The signatories were Dr Michael Ashburner of the University of Cambridge, Professor William Shaw of the University of Leicester and Dr David Glover of Imperial College, London. Their concern arose from a feeling in the research community that scientists have to waste too much time in writing or modifying programmes that are system-dependent. It was also felt there was scope for an improvement in access to databases.

Research council officials are at pains to emphasize that no financial commitment is implied by next month's meeting. Enthusiasts in the research community, however, are already talking fondly of a network of "maybe 6 or 8" VAX machines at national centres, providing regular updates for databases and allowing the transfer of the latest software. These might in turn be connected to microcomputers for local analysis once the letter crunching had been done by the large machines. One estimate puts a price of "one or two million" pounds on the scheme.

The three research councils involved, the Medical Research Council, the Agricultural and Food Research Council (AFRC) and the Science and Engineering Research Council, all have their own budgetary problems. But, it is pointed out, all of these bodies give a high priority to the new biology, and the cancer charities are thought likely to look favourably on the idea. One of the difficulties is that the subject falls between the concerns of several research councils; however, the existence of the inter-research committee, whose chairman is Dr John Ingle of AFRC, has allowed a quick response. And, it is argued, the advantages of a computer link-up have already been demonstrated by the "Starlink" network used by astronomers.

Tim Beardsley

Peace protests

Soviet missiles unwelcome

THE Czech human rights organization, Charter-77, two weeks ago formally repudiated views it alleged had been ascribed to it by the British-based "European Nuclear Disarmament" movement (END). Peace, said the Chartists, is not in itself a sufficient goal — it may mean little more than passivity in the face of tyranny. International peace, they said, can be guaranteed only if governments treat their own citizens with respect. Charter-77 is not a peace movement; there is a "long road" from "the absence of war" to "real peace".

The Chartists' statement seems an overreaction to the current Warsaw Pact propaganda which puts the entire blame for the present nuclear confrontation on the United States. The Czechoslovak people as a whole seem reluctant to accept the official line. Party activists have reported difficulty in convincing factory meetings of the essential difference between "aggressive" United States missiles and their Soviet counterparts now being installed in Czechoslovakia to "defend peace".

Many have signed petitions against Soviet missiles, and students at the Charles University in Prague, aware that signing the petition would almost certainly result in expulsion, devised their own form of protest; a huge placard condemning the Soviet missiles was set up near the entrance to the university, with an invitation to all who agreed with the message to draw a little Sun on it. Within a couple of hours, the whole board was reportedly covered in Suns.

There is now evidence that concern about the Soviet missiles is widespread throughout the bloc. In East Germany, which is also to accommodate the Soviet missiles, there is a well-established "independent" peace movement. This accepts the concept of the bilateral withdrawal of missiles (and later of conventional arms), but is not acceptable to the authorities.

And in both Hungary and Bulgaria, the governments have denied widespread rumours that Soviet missiles may be arriving in the near future. The Bulgarians are particularly incensed, as they are committed to the campaign for a Balkan nuclear-free zone. In Romania (except when Soviet visitors are present), even official spokesmen take the line that they cannot apportion blame, albeit with hints that the West must make the first move. No "unofficial" opinions have so far been reported.

Poland has so far contributed little to the missile debate — save for the usual spate of "spontaneous" condemnations of the West. At least one group, however, has refused to sign such a document (see *Nature* 22 March, p.305). **Vera Rich**

Toxic chemicals

UK guidelines a compromise

BRITAIN's Health and Safety Executive seems to have pleased nobody with its first attempt at British guidelines on occupational exposure to toxic substances, published last week under the unassuming title "Guidance Note EH 40". Some of the concentration limits specified are admitted by industry not be be achievable in the foreseeable future, while trade unions are complaining that the new document weakens their members' legal protection.

Until last week, Britain relied largely on the toxic chemical guidelines produced annually by the American Conference of Government Industrial Hygienists. These were used by factory inspectors to assess compliance with the statutory requirement on employers to ensure their employees' health, safety and welfare "so far as is reasonably practicable". But differences of practice between Britain and the United States, and the conflicting requirements of European Community directives, made home-grown guidelines desirable, and consultations were started in 1980.

The main change is that short-term exposure will in future be assessed over a 10-minute period, rather than (impossibly) instantaneously. This has pleased those who worry about the theory of occupational hygiene, even though, for substances with very rapid effects, the 10-minute principle will have to be ignored. But the numbers in the new list are copied wholesale from the US list it was intended to replace.

These "recommended limits" are "considered to represent good practice" and will be used by factory inspectors "as part of their criteria for assessing compliance". The Health and Safety Executive explains that there are not enough toxicity data on most substances to warrant "control limits", which have been drawn up for fewer than a dozen materials. Control limits for these materials have been set after prolonged haggling between employer and union representatives on the executive's Advisory Committee on Toxic Substances. Embarrassed by the shortness of the list of control limits, executive officials stress that EH 40 will be updated regularly.

Control limits, as distinct from recommended limits, are considered to be "reasonably practicable" and "should not normally be exceeded", according to the executive. But Mr Edward King, an ex-president of the Institute of Occupational Hygienists, says that the existing control limited for lead could not be achieved at any secondary lead smelting factory in Britain in the forseeable future without unrealistic expense. The Chemical Industries Association reluctantly concedes that its members may have difficulty in reaching the supposedly enforceable control limit within 2 years. The limit for acrylonitrile may present similar difficulties.

The Association of Scientific, Technical and Managerial Staffs (ASTMS) condemns EH 40 for being out of date, based as it is on the US list for 1980, which has since been updated. Others think this is no bad thing. ASTMS also complains that it has not been given documentation of the new limits — although there does not seem to be any new scientific work underlying EH 40 and the union is represented on the committee that fixed the limits.

So what is the professional occupational hygienist's considered view of EH 40? Mr King has no hesitation in describing it as window-dressing that will do nothing to improve anyone's health. He believes that most damaging exposure to toxic industrial chemicals now occurs during cleaning and maintenance work, when normal codes of practice are often informally suspended in any case. Real improvements will have to wait until factory inspectors are more numerous or can impose more effective sanctions than at present. **Tim Beardsley**

Modest ecological disaster discussed

Washington

WHAT is fondly known as the "doom and gloom" lobby — the environment and world-future think-tanks that have been warning us for years about overpopulation, deforestation, acid rain, soil erosion and sundry depletions of non-renewable resources — met in Washington last week to say that things are not so bad after all. A conference organized by the World Resources Institute (a new "Center for Policy Research" in the Brookings Institution mould that is also known as the Council-on-Environmental-Quality-in-exile, thanks to the Reagan Administration's efforts to do away with that agency) entitled "The Global Possible" concluded that while the "doom and gloom" scenarios "could be valid", they were "confident that these trends can be reversed". How? By holding the world population to 8,000 million, arranging for a smooth transition to non-fossil fuels, controlling tropical deforestation, avoiding a disruption of global climate, increasing agricultural production, decreasing soil erosion, and, last but not least, strengthening public support for sound resource management.

Among the participants were Russell Train of the World Wildlife Fund, Lester Brown of the Worldwatch Institute, Martin Holdgate of the UK Departments of Environment and Transport and Mihajlo Mesarovic of Case Western Reserve University, a founding member of the Club of Rome. Stephen Budiansky

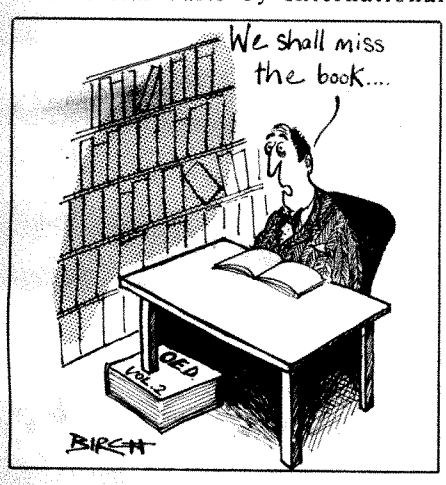
Computerized dictionary

English language quantified

THE *Oxford English Dictionary*, generally known as the OED, plans to leap into the twentieth century by investing £7 million in the computerized production of a new edition, a merger of the existing 12-volume dictionary with the four-volume supplement still not yet complete. Announcing this plan earlier in the week, Oxford University Press said that the merging of the OED and supplement into a single printed work (to be published in 1988) is merely the first phase of an ambitious programme leading to the availability of the dictionary in an electronic form and eventually to the use of computers for novel lexicographical purposes.

Oxford seems to have been able successfully to turn the respect and affection in which the OED is held into tangible support for the computerization project. IBM (UK) Ltd is lending for the first phase of the project computer hardware (a 4341 processor and up to a score of terminals), software and the services of two specialists on secondment. This assistance, valued at £1 million over the duration of the first phase, compares with the £6.5 million in cash and kind committed in a year by IBM to the support of computer development projects in the United Kingdom.

The Department of Trade and Industry has also provided a grant of £288,750 from its fund called Support for Innovation. The donkey work of "data capture" (or keyboarding) will be carried out on a commercial basis by International



Computaprint of Fort Washington, Pennsylvania.

In preparation for the later phases of the project, Oxford has also made an arrangement with the University of Waterloo, Toronto, Canada, which has undertaken to develop software for the computerized handling of lexicographical data. One obvious possibility is that the dictionary, supplemented by recently accumulated material, will be made available on magnetic tape or by means of a data bank, but there is also a possibility of making the whole work available on optical disks, one of which might be sufficient to contain the 1,000 million characters in the new edition. Oxford will pay a royalty for its use of the software, which the University of Waterloo will be free otherwise to exploit.

For users of the dictionary, the chief disappointment will be that the new edition, to be called the *New Oxford English Dictionary*, will be innocent of the entries accumulated since the appearance of the first supplementary volume nearly twenty years ago. Keyboarding will begin in September this year, but between now and then the dictionary staff will be chiefly concerned to develop the means by which the entries in the OED and the four supplements can be accurately logged and merged, given the variety of typefaces and alphabets used and the need for subjective judgement in telling how supplemental entries supplement the originals.

As things are, the project is cautious about the extent to which the resource being computerized may be used as an automatic source of novel dictionaries, but there seems a good chance that scholars will eventually be able to use the dictionary as a means, for example, of monitoring the rate at which nouns are being converted into adjectives or even verbs. □

Nuclear power

UK peers give Europe advice

THE European Commission should play a stronger role in environmental and energy policy-making, according to a new British report from the House of Lords Select Committee on the European Communities. The committee concludes there is a clear need for an authoritative international assessment of safety and other environmental aspects of nuclear power aimed at reassuring public opinion. It suggests that the commission might join forces with a body such as the International Energy Agency, in order to avoid the appearance of making propaganda.

In the meantime, it suggests that European reliance on the pressurized water reactor could have the "grave disadvantage" that a serious fault or an accident in one of these reactors might cause public opinion to swing against the nuclear energy programme in general. Because of the need to support diverse energy sources, their lordships are concerned to protect and encourage the role of nuclear power, so they suggest that the EEC should give serious consideration to advanced gas-cooled reactors (AGRs) as a possible future design. One of Britain's AGRs, at Hinkley Point in south-west England, is described as "a model of efficiency".

The House of Lords Committee reserves some scornful words for the present policies of the European Commission's Directorate-General XI, which concerns itself with environment, consumer protection and nuclear safety: "Public concern — well-intentioned but not always well-informed — leads to regulatory action before the relevant facts have been established". The Commission directive on sulphur emissions is cited as an example.

The general European objective of reducing oil imports is endorsed, however. The committee sees a danger that lower oil prices at present, which it sees as only temporary, might provide member states with an excuse to delay the move away from dependence on oil. It is also worried by the prospect of greatly increased imports of coal into the EEC, which are thought likely to be necessary in the 1990s. It therefore says that "profitable home production of coal should be encouraged", a delicately-worded statement that might be taken up by either side in the current British miners' strike.

Tim Beardsley

AFRC cuts meet disapproval

BRITISH Members of Parliament have taken up the cause of scientists employed by the Agricultural and Food Research Council who are threatened by redundancy. A report from the House of Commons Agriculture Committee published last week chides the government for imposing on the council cash limits that have forced a programme of contraction.

The committee's predecessor in the last parliament recommended a shift towards more food research, a move since implemented by the council. But the new committee now protests that it was never intended that this should be at the expense of mainstream agricultural research.

The council's submission to the Advisory Board for the Research Councils on scientific opportunities for 1985–86 includes a similar request for more provision for food science without this being at the expense of other work. Plant biotechnology is another area where the council wants to see increased provision.

The government's response to last year's Agriculture Committee report was to propose a new priorities board to advise on the strategy for scientific research related to agriculture, though in the words of Mr Michael Jopling, the Agriculture Minister, "it is expected the advice will usually be followed". But this does not satisfy the Public Accounts Committee, which complains that agricultural research is still "dangerously poised between many pinnacles of bureaucracy". Tim Beardsley

Nuclear war models

SIR — Our study of frost rings as indicators of climatically effective volcanic eruptions (*Nature* **307**, 121; 1984) was discussed by John Maddox in the same issue (p.107) in the context of recent calculations modelling the long-term consequences of nuclear war. We appreciate his synopsis of our study but we do not necessarily agree with his conclusions on the applicability of the frost ring-eruption relationship to the "nuclear winter" question. Mr Maddox emphasized the formation of a frost ring as evidence of a short-lived climatic response to a major eruption and contrasted this seemingly moderate reaction to an atmospheric aerosol veil with the severe picture presented in the nuclear winter scenario, attributing the disparity between the two responses to the nature of the assumptions made about the quantities of dust carried into the stratosphere.

Frost rings are most likely to form at the end of a summer that is already notably or even abnormally cool. Cold delays the completion of cambial activity, rendering the tree more vulnerable to damage by late summer polar-air outbreaks, which occur while the tree is still growing. This suggests not only a short-term regional climatic response, in the form of an unusual incursion of cold air over a period of a few days in years following a major eruption, but also reflects a longer-term response of general cooling — an extreme example of which seems to have occurred in the North-Atlantic sector in the "year without a summer", following the eruption of Tambora in 1815. If a single point source of atmospheric aerosols injected into the stratosphere can have such significant apparent short-term and long-term effects on climate, as well as a characteristic biological response, we believe the implications for the response to global nuclear war are grave indeed.

Nevertheless, the volcano-climate link is appropriate as an analogue for only that portion of the nuclear-war scenario that occurs as a result of large amounts of dust and aerosols reaching the stratosphere . One should indeed expect a large disparity between the respective responses of the climate system to the aftermath of a major volcanic eruption and the aftermath of a global nuclear war, as the former is a reaction to conditions primarily in the lower stratosphere while the latter is likely to be a far more complex response to conditions through several layers of the entire atmosphere, the most important of which is probably cooling due to heavy concentrations of soot and smoke in the mid-troposphere. Mr Maddox seems to have missed the essential point repeatedly made by Turco *et al.* that smoke, because of its high absorption in the visible light range and low absorption in the infra-red, causes most of the surface cooling.

Thus the "Krakatoa effect" — namely, a climatic and biological response to an eruption's aerosol veil — certainly supports the likelihood of a similar effect a nuclear war, but that the volcano-climate relationship alone is not wholly analogous to the far more complex and much more severe climatic and biological responses that Turco *et al.* (*Science* **222**, 1283; 1983), Ehrlich *et al* (*Science* **222**, 1293; 1983) and Covey *et al.* (*Nature* **308**, 21; 1984) have postulated.

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● The point of that part of the article complained of was to object that one of the principal references was then still "in preparation" and to assert that predictions of nuclear winter should be regarded as qualitative until more accurate data and calculations are available — Editor, *Nature*.

Lie detector lies

SIR — It is illuminating to express the results of studies in the fallibility of lie detection, such as that of Kleinmutz and Szucko[1], in terms of the amount of information obtained, which can be measured as the "expected weight of evidence" introduced by I.J. Good[2]. Doing so reveals that what is obtained is a small fraction of the information required to take any rational decision.

Expressing experimental results as proportions: valid positive: proportion p of all actual liars, valid negative: proportion q' of all truth-tellers, false positive: $p' = 1 - q'$, false negative: $q = 1 - p$. The expected weight of evidence is the expected value of the logarithm of the Bayes' factor, which is

$$I = p \log (p/p') + q \log (q/q')$$

The logarithms can be taken to any base desired, and I follow Good[2] in using logarithms to base $10^{0.1}$ and calling the result "decibels" (db). Strictly, due allowance should be made for the small sample size, but for illustration I shall use the crude proportions as quoted by Kleinmutz and Szucko, which will tend to overestimate I. The value of I obtained for the results of their six interpreters are 1.3, 0.6, 2.8, 2.1, 0.8, 1.3, averaging 1.5 db (the variation is probably random rather than a reflection of varying skill). This is a very small amount of information. To make a confident decision in normal circumstances we usually need some 20 db — for instance, if we start with a hypothesis at "evens" (50 per cent probability), a log-factor of 20 db, that is a Bayes' factor of 100, will make it 100 to 1 on (99.01 per cent probability), which can perhaps be regarded as "practical certainty". In some proposed applications of the lie detector, however, we are starting with an *a priori* improbable hypothesis — that an apparently honest person is a traitor, for instance. If the prior odds are estimated as 1,000 to 1 against, the log-factor required for practical certainty becomes 50 db.

If some procedure can be devised for collecting many independent scraps of information like this and combining them accurately, for instance by estimating credible log-factors and adding them, it might be possible to produce a reliable result. The "guilty knowledge test"[3] which Kleinmutz and Szucko refer to is an attempt to do this: Lykken suggests using 10 to 16 independent items, which might add up to from 15 to 30 db. Where only the result of a single lie-detector test is available, the "confirmation" that it provides is so small that the only just course is to disregard it entirely.

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1. Kleinmutz, B. & Szucko, J.J. *Nature* **308**, 449-450 (1984).
2. Good, I.J. *Probability and the Weighing of Evidence* (Griffin, London, 1950).
3. Lykken, D.T. *J. appl. Psychol.* **44**, 258-262 (1960).

Crater dated

SIR — The cover of your 19 April issue shows an aerial photograph of Meteor Crater, Arizona. It is stated in the caption that the crater is the "result of an impact about 50 million years ago" but I am unaware of any Meteor Crater age estimates of this magnitude. The crater is clearly of Pleistocene age.

The age of Meteor Crater has been estimated using various lines of reasoning. It was observed 50 years ago that sediments at the crater show evidence of the Wisconsin glacial episode[1]. These soils were later correlated with the well-studied stratigraphy at Hopi Buttes and it was observed that a Pleistocene sediment directly overlies the crater ejecta[2]. Two major pluvial episodes of Pleistocene age are recorded as distinct erosional patterns within the crater[3].

I have recently applied thermoluminescence (TL) dating to shock-metamorphosed sedimentary rocks from Meteor Crater (in preparation). Meteor Crater can be dated by the TL method because the TL of the target rocks was reset by the high temperatures experienced during the meteorite impact. After cooling, TL reaccumulated due to exposure to natural radioactivity. These measurements indicate that the Meteor Crater impact occurred 49,900 years ago with an estimated uncertainty of 2,900 years.

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1. Blackwelder, E. *Science* **76**, 557-560 (1932).
2. Shoemaker, E.M. in *The Solar System* (eds Middlehurst & Kuiper) 301-336 (University of Chicago Press, 1965).
3. Shoemaker, E.M. & Kieffer, S.W. *Guidebook to the Geology of Meteor Crater, Arizona* (Center for Meteorite Studies, Tempe, Arizona 1979).

Tropical ecology research

SIR — The table below is compiled from the monthly listings of ecological publications in *Current Advances in Ecological Sciences* between 1979 and 1983. The journal (published by Pergamon, Oxford) cites an annual average of 15,000 recent ecological publications in about 2,000 source journals worldwide, under 56 major categories which I have condensed to 14 topics. The table shows a declining annual trend in tropical ecological research publications from 2.93 per cent of the global total for ecological publications in 1979 to about 1 per cent in 1983. The decline is also evident in each of the 14 areas listed except for tropical ecology (*sensu stricto*).

The major areas of research shown in the table are: (1) plant and animal interaction covering host/parasite relationships, competition and interaction of man and microorganisms (24.2 per cent); (2) plant and animal communities — sociology, population dynamics and behaviour (14.0 per cent); and (3) growth, diseases and adaptation (11.3 per cent). Very little (0.2–2.4 per cent) tropical research work was done in (1) micrometeorology and the use of artificial environments for studying the functions of tropical plants and animals; (2) water relations and hydrology; and (3) the ecology of various tropical plant communities as ecosystems.

The above trends indicate that tropical ecological research has been utilitarian, with emphasis on topics of direct benefit to mankind especially for food production. However, in view of the spread of desertification — which now affects 34 countries in Africa — and the economic crisis caused by the prolonged drought during 1982–83 in more than 24 African countries, including some coastal countries in the wet tropics, I think that much more emphasis should be placed on tropical ecological research dealing with water relations, hydrology and climate.

What is responsible for the decline in tropical ecological research publications? Can it be due to the effects of the economic recession and inflation on research work at universities and governmental institutions in developing countries in the tropics? One trend which has been obvious during the period is the massive migration of seasoned university staff and scientific research workers from the poorer developing countries in Africa to the more affluent developing countries in Africa as well as to the industrialized countries outside the continent. This brain-drain may have contributed to the decline in ecological research work in the tropics; other factors may be the paucity of research funds and facilities, and of the trained middle level manpower needed to support field work.

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Trends in tropical ecological research 1979–83

	1979	1980	1981	1982	1983	Total (%)
General tropical ecology (historical, biogeography, dynamics of ecosystems)	43	38	16	16	10	123 (9.5)
Plant and animal communities	57	41	43	16	25	182 (14.0)
Productivity and energy	29	16	10	7	9	71 (5.5)
Plant/animal interactions	111	94	55	22	31	313 (24.2)
Biogeochemical cycles and nutrition	18	11	11	9	6	55 (4.2)
Water and hydrology	5	4	4	5	3	21 (1.6)
Growth, diseases and adaptation	51	45	26	14	10	146 (11.3)
Autecology, genecology and crop ecology	29	30	18	12	10	99 (7.6)
Micrometeorology and artificial environment	1	1	0	0	0	2 (0.2)
Soil biology, erosion, humus and microbes	22	20	5	7	6	60 (4.6)
Environmental problems (hazards, conservation, wildlife, pollution, weed and pest control)	26	22	26	13	21	108 (8.3)
Land use, surveys, remote sensing and statistical sampling	16	8	6	3	7	40 (3.1)
Tropical ecology	2	0	4	18	21	45 (3.5)
Forest, grasslands, freshwater and microbial ecology	5	9	11	5	1	31 (2.4)
Total entries for the tropics	415	339	235	147	160	1,296 100
% Of total entries (all subjects)	2.94	3.59	1.97	0.62	1.09	- -

Classic errors

SIR — R. H. Wright and R. E. D. Cattley (*Nature* **303**, 568; 1983) and M. Macnamara (*Nature* **307**, 590; 1984) discuss the possible meaning of Homer's "wine-dark" sea. Wright and Cattley propose a chemical answer to the problem, suggesting that wine when mixed with the alkaline water of the Peloponnese would turn blue. Macnamara, following W. B. Stanford's observation that the Mediterranean can look wine-red, assumes that the primary question is to account for the redness of the sea, and makes the extraordinary assertion that Homer "mentions only red wine in his epics".

Wright and Cattley have not faced up to the implications of the Ionian origins of Homer and much of Greek culture up to at least the seventh century BC. What happened in the Peloponnese may be irrelevant compared with what happened in Asia Minor.

And (*pace* Macnamara) wine in Homer can be black as well as red. At *Odyssey* 5.265 and 9.196 we meet the phrase *ασκον...μελανος οινοιο*, "a wine-skin of dark (black) wine". Thus "wine-dark sea" may mean a black sea no less than a red sea.

The Homeric adjective *οινοψ* (wine coloured) occurs in the variant forms *οιωψ* in Sophocles and *οινωπος* in other Greek writers. At *Iliad* 18.562 we find *μελανες...βοτρυες*, ("a bunch of black grapes"); similarly, Sophocles (*Oedipus at Colonus* 674-5) applies *οινωψ* to ivy-berries. In fact wine was often described as "sparkling" in Homer (see *Iliad* 1.462 and 4.259), and the particular element of the sea which is important in the Homeric *οινοπα ποντον* is the sparkle of light reflected in the otherwise dark sea. Thus Theophrastus of Straton (*De Coloribus* 2.8) uses *οινωπος* to mean "black mixed with bright light". It may be (as Stanford believes) that *οινοπα ποντον sometimes* refers to a red sea. But this does not have to be the case, and to concentrate exclusively on the redness is to miss the real point. The sea is seen as a dark force, menacing and unpredictable (compare the primary meanings of the verb *πορφυρω* and the adjective *πορφυρεος*), and in this, as in wine, the light and the Sun are reflected (in the words of the poet) "in daggers of bright gold".

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Yellow rain — a palynological analysis

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Yellow rain, purported to be an agent of toxin warfare in South-East Asia, contains a high percentage of pollen. Analysis of this pollen in yellow rain samples suggests that they are the faeces of honey bees.

A HIGH pollen content has been reported in samples of yellow rain from South-East Asia[1-7]. We have examined 11 samples of yellow rain allegedly collected at sites of chemical attack in Laos. The samples contain a high proportion of pollen. No two samples, even different spots of yellow rain on the same leaf, have the same composition of pollen types. The plant taxa identified are common in South-East Asia. Seven pollen types in yellow rain samples were also found in *Apis cerana* and honey from Thailand. In general appearance, high pollen content, and differences in pollen composition from one spot to another, the yellow rain samples resemble honey bee faeces.

The first two yellow rain samples we analysed were from small reddish-brown rocks with yellow spots. The third, the ABC News sample, is reported to have been scraped from vegetation by Hmong soldiers in Laos in March 1981 and to contain three trichothecene mycotoxins[8]. The remaining 8 samples were yellow spots from the upper surfaces of narrow lanceolate leaves about 15 cm long. The rocks and leaves were given to Canadian Embassy officials in Thailand in April 1982 by Hmong refugees who said they had collected them at two sites of chemical attack in Laos in March. The records do not specify whether the rock and leaf samples we examined are from one or both sites. All spots were 2–5 mm in diameter, agreeing with earlier descriptions[1,2,7,9]. A 3 mm spot from a leaf weighed 1.5 mg and contained 1.1×10^5 pollen grains. We examined pollen from abdomens of two Asian honey bee species, *A. dorsata and A. cerana*, collected in Thailand by T.D. Seeley, who first suggested that yellow rain samples from South-East Asia might be faeces from cleansing flights of honey bees[7]. We also tested two samples of honey purchased in July 1983 at villages on the Thai-Laotian border, and known faeces of *A. cerana* and *A. dorsata* on leaves collected in July and September, respectively, near Poona, India.

The first rock sample (Table 1, rock 1) was the residue on a piece of paper on which the rocks had been examined. This, and all other samples except as noted were acetolysed before scanning electron microscopy (SEM)[10]. It contained at least six pollen types. None could be confidently identified even to family. The most numerous type, triaperturate and 20–23 μm long, is designated Fagaceae-like. The second sample, rock 2, was from a single spot. Its pollen consisted almost entirely of one of the minor types in rock 1, plus some grass pollen.

The ABC News sample consisted of four yellow fragments of approximately 2 mm^3. SEM of an untreated fragment revealed vague outlines of pollen in an unknown matrix (Fig. 1*a*). Another untreated fragment, mounted in glycerine jelly for light microscopy, appeared to consist mainly of pollen, plus a few bee hairs (setae). The remainder of the sample was acetolysed, revealing about 20 pollen types, some of which are shown in Fig. 1*b, d*. Families identified were Compositae, Dilleniaceae, Elaeocarpaceae, Euphorbiaceae, Icacinaceae, Myrtaceae, Polygalaceae, and Sterculiaceae (Table 1). There was also a small proportion of grains possibly of Melastromataceae/Lythraceae. Grains were found that are indistinguishable from *Dillenia pentagyna* taken from herbarium specimens. Also found (Fig. 1*d*) were grains indistinguishable from those of *D. hookeri*, a species common in Laos[11]. Another type in the ABC News sample (Fig. 1*d*) is indistinguishable from pollen from herbarium specimens of *Macaranga denticulata*, although its rather unspecialized morphology and the existence of some 300 species of *Macaranga* make narrow identification difficult. The most common grain in the ABC News sample is very small, about 10 μm, with three apertures and a smooth/irregularly-pitted surface (Fig. 1*d*). It is classified as Elaeocarpus-like. Also, fungal hyphae and a few setae were seen in acetolysed preparations of the ABC News sample.

Leaf samples 1 and 2 were spots from different leaves, while 3A and 3B were spots a few cm apart on a single leaf, as were the sample pairs 4A/4B and 5A/5B (Table 1). Leaf samples 1 and 2 had three pollen types present in the ABC News sample — *M. denticulata*, Melastomataceae/Lythraceae and a pollen type tentatively placed in *Apodytes* (Icacinaceae). In addition there were hyphae, setae, and stellate, trichome-like structures. An abundant grain in leaf sample 1, probably from Moraceae/Urticaceae (Fig. 1*e*), was not seen in leaf sample 2. Conversely, an abundant grain in leaf sample 2, from *Ilex* (Fig. 1*f*), was not found in leaf sample 1.

Leaf sample 3A contained *M. denticulata, Ilex, Apodytes*, Melastomataceae/Lythraceae and a small proportion possibly of *Nuphar* (Nymphaeaceae). Sample 3B consisted mainly of Fagaceae-like grains, matching those in rock sample 1. The remaining leaf spots 4A, 4B, 5A and 5B, all had *Ilex, M. denticulata, Apodytes* and Melastomataceae/Lythraceae, but in very different propor-

Table 1 Summary of pollen identifications

	YELLOW RAIN											
	Rock 1	Rock 2	ABC News	Leaf 1	Leaf 2	Leaf 3A	Leaf 3B	Leaf 4A	Leaf 4B	Leaf 5A	Leaf 5B	*A. cerana*	Honey 2
AQUIFOLIACEAE *Ilex*					++	+		*	*	*	*		
COMPOSITAE			*										
DILLENIACEAE *Dillenia pentagyna* *Dillenia hookeri*			+ *										* *
EBENACEAE Diospyros-like												++	
ELAEOCARPACEAE Elaeocarpus-like			++										+
EUPHORBIACEAE *Macaranga denticulata*			+	++	++	++		+++	+	++	++		
FAGACEAE-LIKE	++						+++						*
GRAMINEAE		+										*	
ICACINACEAE *Apodytes*			*	++	++	+		*	+++	+	*		
MELASTOMATACEAE/ LYTHRACEAE			*	+	+	+		+	+	*	+		*
MORACEAE / URTICACEAE				++									
MYRTACEAE			*										*
POLYGALACEAE			*										
SAPINDACEAE													++
STERCULIACEAE *Sterculia*			*									+	
UNIDENTIFIED, 35-38μ 4 Apertures			+										*
UNIDENTIFIED, 25-36μ Punctate, 3-Colporate	+	+++											
UNIDENTIFIED, 45-50μ Thick Exine										+			

Key: *, minor component (<5%); +, 5–10% of sample; + +, 10–50%; + + +, more than 50% of samples.

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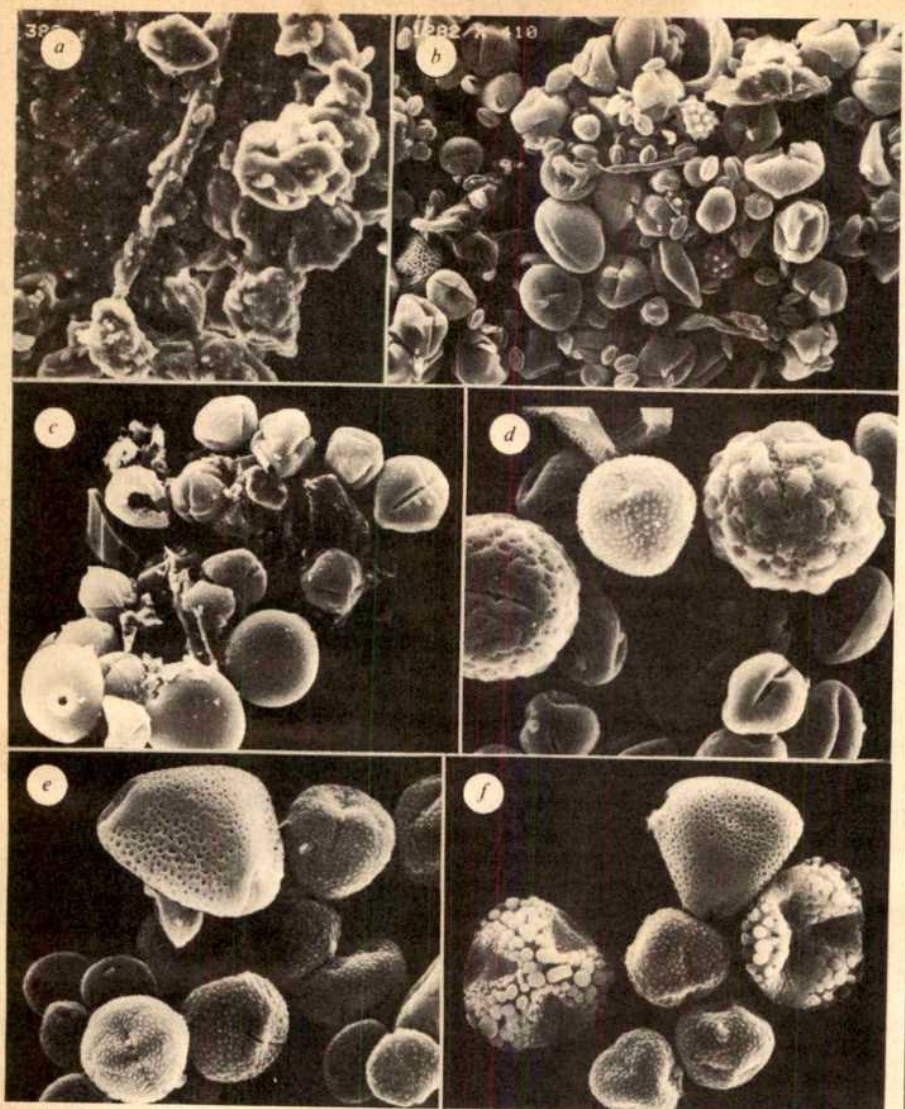


Fig. 1 SEMs of pollen in yellow rain. *a*, ABC news sample, untreated, X250. *b–f*, All acetolysed preparations. *b*, ABC News sample, X270. *c*, Rock sample 2, three spheroidal grains at bottom left are from the grass family, X330. *d*, ABC News sample, larger grain at upper right and partial grain middle left are probably *Dellenia hookeri*; grain between is *Macaranga denticulata*; the triangular grain in background is probably Myrtaceae; numerous smaller gains were classified as Elaeocarpus-like, X1,300. *e*, Leaf sample 1, the largest grain, seen in oblique view is assigned to *Apodytes*; the smallest grains, some showing pores, are Moraceae/Urticaceae; the remaining ones with furrows are *M. denticulata*, X1,170. *f*, Leaf sample 2, the uppermost triangular grain is *Apodytes*, the three small grains are *M. denticulata*, and the remaining two are *Ilex*, the one on the right is partially collapsed, X980.

tions in each case. In addition, 5A had an unidentified type 45–50 μm long, 3–4 aperturate with a thick exine, that was not found in the other samples. The great diversity of the ABC News sample as compared with the leaf and rock samles may be a consequence of collection technique — a number of yellow spots may have been combined.

An abdomen of *Apis cerana* collected in March 1980 contained at least three pollen types: Diospyros-like, a small proportion of grains from the grass family (Gramineae) and a reticulate type 35 μm long, matching in form and range of variation a type in the ABC News sample tentatively assigned to *Sterculia*. *A. dorsata*, collected in March 1980, had pollen probably representing two or three closely related species in *Castanopsis/Lithocarpus* (Fagaceae).

Honey sample 1, from Nakhon Phanom (17° 25′N: 104° 45′E) had at least ten pollen types, but none matched those in the yellow rain samples. Honey sample 2, from Chiang Khan (17° 55′N; 101° 45′E) contained many pollen types including Fagaceae-like, seen in rock sample 1 and leaf sample 3B; the type designated Melastomataceae/Lythraceae, found in the ABC News sample and in leaf samples 1, 2, 3A, 4A, 4B, 5A and 5B; and four more types seen in the ABC sample: *D. pentagyna*, Elaeocarpus-like, and an unidentified four-aperturate grain, 35–38 μm long.

Known faeces of *A. cerana* and *A. dorsata* on leaves collected near Poona, India resembled spots of yellow rain in size, general appearance, high pollen content and the presence of setae and hyphae. Each of three spots of *A. cerana* faeces had at least five pollen types, with only some common to all three. Faeces of *A. dorsata* from a nearby site showed similar diversity from spot to spot.

Our identifications of pollen in yellow rain samples were compared with those in a report from the Australian Defence Department[2]. One of their three samples had mainly Harpullia-like pollen (Sapindaceae); the other two were predominantly Rapanea-like (Myrsinaceae). A Chinese study describes a "yellow rain" phenomenon in September 1976 in Jiangsu[12]. Occurrences generally lasted a few minutes, covering areas of 0.5–20 acres with 2–6mm yellow spots rich in pollen. The authors concluded that the spots were honey bee faeces. Mass cleansing flights of *A. melifera*, causing similar phenomena, have been studied previously[13,14].

All of the plant taxa we identified in samples of yellow rain are common in South-East Asia. The three assignments we made at the species level, *D. hookeri, D. pentagyna* and *M. denticulata*, are trees native only to Southern Asia. Also, a reticulate type in the ABC sample assigned to *Sterculia* was present in *A. cerana* collected in the same season in Thailand. Most significantly, honey sample 2 contained six pollen morphotypes we found in yellow rain, showing that these pollen types are gathered by honey bees in Indochina. No two samples of yellow rain we examined had the same pollen composition; even adjacent spots on the same leaf were different. Pollen diversity from spot to spot was also found for known bee faeces from India. Such differences between spots would not be expected if the spots of yellow rain had been disseminated by an artificial source. Our observations lead us to conclude that the yellow rain samples we examined are probably honey bee faeces.

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Natural history of yellow rain

The discovery that some of the yellow rain falling from the sky in South-East Asia is the excrement of bees, first dismissed as impropriety, must now be taken seriously.

REPORTS in the past few years that mycotoxins have been used as lethal weapons, in South-East Asia and elsewhere, are not formally disproved by the preceding article which demonstrates that at least some of the yellow-coloured droplets falling from the sky are nothing but the excrement of bees. But they are a serious challenge for those who have been arguing that visual observations of "yellow rain" can all be counted as evidence of the use of chemical weapons against embattled populations, usually of civilians. And because the cause for supposing that yellow rain is proof of the use of mycotoxins against people has not significantly strengthened since the then US Secretary of State Alexander Haig gave it wide currency in a speech in West Berlin three years ago, there is the strongest possible reason why the scraps of evidence which survive should be looked at again, and reassessed.

Reports of the use of chemical agents against people in South-East Asia, principally in Laos and Cambodia (now Kampuchea) have been current since the late 1970s. By the time the United Nations set up a committee to investigate the allegations, towards the end of the General Assembly in 1980, there was a great mass of untested anecdotal evidence, usually gathered from the verbal reports of refugees from the endemic conflict in that part of the world. Many people from villages in the region reported having suffered from rashes, pulmonary and bronchial trouble and fevers after materials of various kinds and colours (black, blue, red, yellow) had been sprayed from the sky by aircraft. Many reported that fellow-villagers had died.

The report of the UN expert committee, which appeared in November 1981, served principally to show how difficult a task it had been given. Permission to visit the areas concerned was never forthcoming, with the result that evidence had to be collected from people already in refugee camps in Thailand and thus unable to point to the places in which supposed toxins had fallen from the sky. The UN expert group confesses in its report that the language problem was a serious impediment to straightforward investigation. While many of the reports thus gathered referred to fatalities in the aftermath of aerial spraying, with other people falling sick, deaths seem never to have been so numerous as to suggest an effective military weapon in the form of a toxic agent.

The suggestion that mycotoxins, toxic materials produced by the metabolism of certain fungal species, might be involved first appeared in a paper issued in Washington by the State Department in the wake of Secretary Haig's Berlin speech, announcing that evidence pointing to the use of toxic agents had been acquired. The identification on the surfaces of leaves smuggled out of South-East Asia of small quantities of trichothecene mycotoxins of a kind produced by species of the fungi *Fusarium* was a considerable triumph for chemists, and for Dr C.J Mirocha in particular. There is no reason to suppose that the measurements are in any way in error, but the manner in which the samples were collected (without controls), and the presumption that "yellow rain" was indeed the vehicle by which mycotoxins had been delivered, is bound to cast doubt on the interpretation of this scanty evidence.

It tends to be forgotten that the investigation of these obscure materials, mostly sesquiterpenes, is not much older than the suggestion that they have been used against human populations. Although it is in retrospect clear that human disease caused by trichothecenes was known to Galen, and while toxic epidemics caused by contaminated grain were common in the Soviet Union in the closing years of the Second World War (and have since been known in the United States, Mexico and India), identifications of chemical structures have all been crammed into the past fifteen years. Analytical techniques, among which the most sensitive are those based on immunological methods, can at their most sensitive detect nanogram quantities. Monoclonal antibodies may bring further improvements.

Pinning down what has been happening in South-East Asia will not be easy, especially if the presumption is correct that mycotoxins have been used in warfare — and if those responsible have changed their ways with all the publicity there has been. The most recent sober assessment of the trichothecene problem, the report *Protection against trichothecene mycotoxins* published towards the end of last year by the National Research Council in Washington (and primarily concerned with the civilian agricultural problem), points out that the symptoms of disease and death reported from South-East Asia are in many ways similar to those experienced by laboratory animals and the handfuls of terminal patients to whom the materials are reported to have been administered. But the report also says that the symptoms are not identical, which may be explained by a mixture of several related mycotoxins as might be expected in natural conditions — or by a quite different cause.

What seems, however, unambiguously to follow from what Nowicke and Meselson have to say is that the association of physical damage to people in South-East Asia can no longer be simply linked with yellow rain. At least some of that consists of bees' excrement, and for the time being there is no evidence that that is associated with mycotoxins. If it were, of course, the suggestion that toxic agents of some kind have been used against people in South-East Asia would be further undermined. But now it seems more prudent that those concerned with investigating the several possibilities should go back to the beginning, and consider other ways in which mycotoxins might have been delivered.

Hitherto, serious questions on these points have been met, at the State Department and at analogous departments of government elsewhere, with the statement that while the publicly available evidence that yellow rain is a shower of pollen doped with toxin so as to make its ingestion more probable may be slight, there is no publicly available evidence that the facts are what they have been said to be. The assumption, indeed the assertion, is that intelligence services know to be a fact what seems in the public world to be improbable. Such arguments nonplus.

For all of us, the best and simplest solution may be that the association between yellow rain and the injury or death of people in South-East Asia should cease to be a lively issue, perhaps as a consequence of climatic or ecological good luck — or perhaps, if people have been using toxic materials in the area, because they change their ways. But since luck is as often bad as good, the only constructive way forward must rely on a thorough investigation of the natural history of yellow rain in South-East Asia, and of the reported association of injury and ill-health among people.

While the State Department has unfairly dragged into a confusing argument evidence that it cannot publish, the governments directly concerned have nothing to lose by letting people investigate what has been happening.

John Maddox

Human evolution

A gathering of our ancestors

from Bernard Wood

THE word 'unique' is abused by us all, but is aptly applied to the 'Ancestors' programme mounted this year by the American Museum of Natural History (AMNH) in New York. Readers will doubtless be aware of the recent resurgence of the creationist movement and its attempts to influence both public opinion and, more importantly, the views of legislators. Scientists have counter-attacked in print but large organizations and institutions have, as always, found it more difficult to respond effectively to what amounts to an attack on the foundations of all scientific endeavour — namely, reason and evidence. The AMNH has not, however, been idle. Three of its curators, John Van Couvering, Eric Delson and Ian Tattersall, conceived the notion of an exhibit of original fossils which would show the public the evidence which enables palaeoanthropologists to trace the path, or paths, that have led to modern humanity. With the endorsement of UNESCO, the museum set about the task of persuading governments, museums and curators to loan their precious fossils. The excellent response — a tribute to the competence and tenacity of the organizers — has produced a spectacular exhibition, which opened to the public on 12 April and will run until September this year.

The designers have sensibly allowed the fossils to tell their own story. The legends are informative, authoritative, but concise, and the fossils have been displayed in a way that allows visitors to see and understand these remarkable documents of our past. Where important specimens were not made available, the organizers have made sensible use of casts to illustrate them. While one can understand the reluctance of curators to part with their fossils on the grounds of security, it is disappointing, but perhaps inevitable, that political considerations should have prevented the display of some material which is supranational in both its scientific importance and its social significance. There are no 'stars' in the exhibit, and all who see it will be impressed, perhaps moved, by different aspects.

What impressed me most were the specimens from the two ends of the time span: the early fossils from the Oligocene and the Miocene, and the late Neanderthals. The enigmatic cat-like skull of *Aegyptopithecus* and the face of *Sivapithecus* are mercifully unaware of how closely their every fissure and prominence is being analysed to seek evidence of affinities. The Neanderthals impress because, despite differences in size and geographical location, they all really do show the characteristic features of the face and vault. They are convincing evidence that morphologists can detect reliable marker features despite the potentially confusing background of intraspecific variation.

Although it is a major part, the exhibit is only one of three components of the Ancestors programme. The two others, a series of study sessions and a five-day symposium entitled 'Palaeoanthropology: the hard evidence', were held last month, and were sponsored by the AMNH with the support of the NSF and the Wenner-Gren and Leakey Foundations. Each study session provided an opportunity for the close study of five or six of the original fossils by as many palaeontologists. My original scepticism of the usefulness of such a scheme was, in large measure, unwarranted. In particular, the sessions made it possible to discuss points of interpretation with those who were expert either on particular anatomical regions, for example Y. Rak (Tel Aviv University) on the face, J. Laitman (Mt Sinai School of Medicine, New York) and W. Kimbel (Cleveland Museum of Natural History) on the cranial base and F. Grine (SUNY, Stonybrook) on the teeth, or on particular remains, such as R. Clarke (University of Witswatersrand) on SK 847 from Swartkrans and Stw 53 from Sterkfontein. These were truly workshops, and ideas were exchanged and debated in a spirit of collaboration which bodes well for future research.

A symposium which attempts a comprehensive review of hominid evolution from the Oligocene to the Holocene is too broad in its scope usefully to address any particular research problem, but three strands could be traced through the five days. The first was the quality and breadth of information about the temporal and palaeoenvironmental context of hominoid and hominid evolution. The second was the increased and welcome emphasis on studying variation within an anatomical region, across a wide range of fossil and extant forms, and the last was the increasing influence of phylogenetic analytical methods on hominid palaeontology.

Reports of research undertaken in the Potwar Plateau (D. Pilbeam, Harvard University), at Laetoli (reported by J. Harris, Los Angeles County Museum), in the Omo Basin (F. Brown, University of Utah) and at the South African Cave sites (C. K. Brain and E. Vrba, Transvaal Museum) served to emphasize the importance of the contrasting, but complementary, contributions of the physical sciences and faunal analysis to evolutionary studies. Brown reported a combination of chemical and microscopic analysis which has demonstrated that an ash widespread in the Omo Basin is present in deep-sea cores taken from the Gulf of Aden. This is compelling evidence in support of the contention that the same ash is present at Hadar (which has been effectively leap-frogged by the marine sediment evidence) and is a powerful illustration of the potential of such techniques. H. Thomas and E. Vrba made thoughtful and impressive use of faunal evidence in their contributions. Thomas reviewed the evidence for two phases of Neogene dispersal across the land bridge(s) between Africa and Asia: one at about 18×10^6 BP and the other at 15×10^6 BP. Vrba urged hominid palaeontologists to see hominids in the context of evolutionary changes occurring in other groups of land mammals. She associated, if not correlated, the marked peak in the rate of appearance of bovid species, and probably other groups as well, with a major change in climate. The apparent appearance at this time of *Homo habilis* and the 'robust' australopithecines suggests that hominids may have reacted in a similar, but more modest, way. It is therefore of some interest that at least one hominid palaeontologist suggested that the early phases of the evolution of *Homo* may have been more complex than usually presented. Vrba's admirable attempts to suggest a scenario for hominid evolution are, like many others, heavily influenced by the assumption that the hominids in Members 4 and 5 from Sterkfontein are taxonomically distinct, but more evidence will be needed before Stw 53, from Member 5, can be confidently included in *Homo habilis*.

There was evidence among some hominid palaeontologists of a move towards considering whether features are widely or narrowly distributed, and utilizing this, and additional, information to deduce morphoclines and thus clade groupings. But the shift towards this philosophy was stoutly resisted by others. Grade and clade arrangements rest on different premises. It is therefore pointless arguing about palaeontological details when at the heart of differences in interpretation are deeper philosophical divisions. Hominid palaeontology must, like other branches of palaeontology, see that its scientific dialogue is adjusted to take account of this. Cladists have to realize that a cladogram based on a narrow range of features stands an even chance of being unreliable when seen against the background of a comprehensive set of character states. Adherents of the classic 'Simpsonian' approach should also be aware that a concept such as the 'total morphological pattern' is often couched in such a general way that any systematic decisions based on it are essentially untestable. □

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Cell biology

Epidermal growth factor receptor—yet another domain?

from Shelley Earp

CELLS responsive to epidermal growth factor (EGF) carry specific receptors for EGF on their surface membrane. Between 12 and 24 hours after EGF binds to these receptors, the cells begin to synthesize DNA. In the meantime, the majority of the EGF–receptor complexes have been removed from the cell surface by a process of internalization into cellular vesicles[1,2]. A long-standing question has been whether the cellular events triggered by EGF are solely a consequence of intracellular messages generated at the cell surface by ligand–receptor binding or whether the internalized complex can have additional intracellular functions. A radical re-examination of this question will be forced by the paper on page 270 of this issue of *Nature* in which Mroczkowski, Mosig and Cohen suggest that the EGF receptor has a direct effect on the structure of DNA[3].

The suggestion stems from the authors' detection of an endonuclease activity in preparations of EGF receptor highly purified by affinity chromatography. The activity is specific for the nicking of double-standed circular DNA and is stimulated by ATP but not by a non-hydrolysable ATP analogue. In those respects it resembles the enzyme DNA topoisomerase II but it has not been possible to demonstrate for the EGF receptor the DNA circle-closing activity characteristic of topoisomerase II[4].

The authors' interest in the latter enzyme, which catalyses topological rearrangements of DNA that are thought to be important in the regulation of transcription and replication[5], may stem from several reports that relate EGF receptor and topoisomerases. First, EGF increases DNA topoisomerase II activity in human foreskin fibroblasts[6] and this activity is also increased during liver regeneration[7], when EGF receptor internalization is known to occur[8]. Second, Wang and colleagues have shown that topoisomerases are covalently bound to the 5′ end of nicked DNA through a phosphotyrosine linkage[9]; the EGF receptor is well known to have tyrosine-specific protein kinase activity and to autophosphorylate its own tyrosine residues. While it is difficult to envisage the same catalytic site both transferring phosphate and forming protein–DNA linkages, the autophosphorylation site on the receptor could also serve as a topoisomerase-binding site.

As the authors point out, an important unresolved question is whether the endonuclease activity of the receptor preparation is the result of a contaminating enzyme detected by a highly sensitive assay. The concentration of receptor used is higher than would be necessary to detect receptor autophosphorylation. Most assays were conducted with 4–16 molecules of receptor per DNA circle and the limit of detection of endonuclease activity was 0.4 molecules per circle. The poor catalytic activity could be due to loss of activity during purification but the nuclease seems to be more stable towards heat denaturation than the receptor's tyrosine-specific protein kinase activity.

The dissociation of nuclease and kinase activity is troubling in another sense. Mroczkowski *et al.* indicate that purified pp60^{src}, the product of the transforming gene of Rous sarcoma virus, has an endonuclease activity similar to that of the EGF receptor. Both pp60^{src} and the EGF receptor exhibit tyrosine-specific kinase activity. If the endonuclease and kinase activities can be dissociated one might expect to see two distinct regions of sequence similarity between pp60^{src} and the EGF receptor. And yet the two proteins seem only to be related by sequence in the kinase domain[10].

Another nucleotide-binding site on the receptor would also need to be invoked to explain the differences in nucleotide specificity of the kinase and endonuclease activities of the EGF receptor — for example, GTP can be used by the former but not latter[11]. The nucleotide specificity of the reported endonuclease activity also differs from that of topoisomerases which, at equivalent concentrations of enzyme and DNA, can be stimulated by non-hydrolysable analogues of ATP[12].

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Given there is little doubt that receptor occupancy causes immediate alterations in membrane function, probably related to stimulation of the receptor's protein kinase acting through tyrosine phosphorylation of perimembrane substrates, autophosphorylation of aggregated EGF receptors, or, as recently proposed, through phosphorylation of phosphatidylinositol[13,14], does the endonuclease activity of the EGF receptor have biological relevance? If it has, receptor internalization must be a prerequisite for EGF action. After a decade of research, the necessity of internalization for the mitogenic response of cells to EGF has not been established — but neither has it been ruled out. So it is worth considering several potentially relevant points about the consequences of internalization.

Since the EGF receptor has been shown to be proteolytically processed upon internalization[15], one possibility is that the process yields a new structure that dictates its own translocation to a specific site of action, such as the nucleus. If the translocated fragment acts catalytically only a small portion of internalized receptor would need to be segregated to the nucleus to produce a biological effect. It may also be of relevance that proteolysis seems to increase the activity and alter the topological specificity of certain nucleases[16]. Perhaps the EGF receptor is a pronuclease whose activity and catalytic repertoire can be increased by proteolysis. If proteolytic activation simultaneously eliminated the binding and antigenic domains of the receptor that are external to the cell surface, the fact that neither EGF binding nor receptor immunofluorescence has been consistently observed in the nucleus could be explained. If the catalytic endonuclease moiety generated by proteolysis had a short half life, it would account for the need to terminate the function it served after an appropriately short time. Any hypothesis that EGF releases a latent endonuclease activity must take into account the fact that EGF does not cause growth in all receptor-bearing cells, that EGF binding and action are influenced by other humoral agents and that in some cells the need for EGF can be abrogated by combinations of other factors[17-20]. It is possible that an endonuclease domain could be processed differentially depending on the cell type or the influence of modulating factors, but would cells opt for another level of complexity in their growth control network?

Several questions raised by the provocative findings of Mroczkowski *et al.* should quickly be resolved. Can the EGF receptor form a phosphotyrosine linkage with DNA? Can conditions be found to express the topoisomerase II-like circle-closing activity? Does experimental proteolytic cleavage of the receptor produce a receptor fragment with greater or more definable endonuclease activity? Is the ATP-stimulated DNA-nicking an intrinsic activity of the EGF receptor or will

a contaminating nuclease be separated from receptor preparations? Cohen has been consistently linked with new advances in the field of growth factors. His pioneering efforts in identifying and purifying EGF and its receptor have led to the discoveries that have set the rest of us in new directions. The new data from his laboratory direct attention to the nuclear effects of EGF and will undoubtedly stimulate discussion and experimentation. However before clamouring to exchange acrylamide for agarose, cell biologists should await further developments. □

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Retinitis pigmentosa

Progress in sight

from Miranda Robertson

THERE is no doubt that molecular genetic technology is leading to an understanding of human genetic disease at a depth and resolution inaccessible to more traditional methods. Because the path to that understanding is often indirect, however, for the time being it presents a problem for clinicians as well as a challenge for research scientists. This is inevitable when the gene defect underlying the disease is unknown, and can be approached only through linked genetic markers. Such is the case in Duchenne muscular dystrophy, for which a marker was first reported two years ago[1], and Huntington's disease, for which a marker was discovered last last year[2]. On page 253 of this issue of *Nature*, Bhattacharya, Wright and their colleagues report an exactly similar marker for the X-linked form of retinitis pigmentosa[3].

As with Duchenne and Huntington's, the retinitis pigmentosa marker is simply an identifiable fragment of DNA that is close enough to the gene to be inherited with it, putting molecular biologists within 'walking' distance of the defective gene itself and offering clinicians the prospect of identifying some symptomless carriers of the gene and, possibly, diagnosing their unborn babies. What are the obstacles confronting the research biologists and the problems facing the clinicians?

Retinitis pigmentosa is the name given to a genetically and phenotypically heterogeneous group of degenerative diseases of the photoreceptor cells of the retina leading, in severe cases, to blindness. The mode of inheritance can be recessive, autosomal dominant or X-linked. The X-linked form, for which Bhattacharya *et al.* now have a marker, is one of the most severe; it accounts for perhaps 30 per cent of all cases and affects about 1 in 20,000 of the population. Because the defective gene is on the X chromosome, of which males have only one, they have no normal gene to compensate for its effects and are severely affected, often in childhood. Females, with two X chromosomes, may have a mild form of the disease or none at all; but they risk transmitting the severe form to their sons. Female carriers of the gene, and the daughters of known carriers, who have no way of knowing whether they themselves are carriers, stand to gain most from the new marker. But it will not help them all, for reasons that follow from the nature of linked markers in general.

The marker in this case is a DNA probe designated L1.28, corresponding to a small fragment of the human X chromosome. What allows this fragment to be used as a marker is its polymorphism — which is to say that it contains minor variations that can be detected by bacterial restriction enzymes (for a slightly fuller explanation of how this works, see ref. 4). L1.28 has two distinguishable variants, A1 and A2. Bhattacharya *et al.* were able to establish the linkage of L1.28 with the retinitis pigmentosa gene on the X chromosome by tracing the inheritance of both the disease and the A1 and A2 variants through five different families.

In the case of close linkage, you would expect the disease to be inherited consistently with one or other of the two variants within a given family; and this is what Bhattacharya *et al.* found. In four families retinitis pigmentosa was consistently inherited with A2; in the other one it was associated with A1. It is just possible that more than one gene on the X chromosome can give rise to retinitis pigmentosa. If so, the linkage will break down in further family studies. So for the time being clinical advice will not be offered on the basis of the L1.28 marker.

But assuming, as seems most likely, that there is only one gene, what are the limitations of L1.28? Most obviously, an affected male relative must be available to establish whether the defective gene is associated with A1 or A2 in the family in question. The second important limitation arises from the fact that both A1 and A2 are present at high frequency in the normal population. Take the case of a young woman whose father has the disease. Since one of her two X chromosomes must be her father's, and his single X chromosome has the defective gene, she will be an obligate carrier and there is an even chance that any son born to her will inherit retinitis pigmentosa. As things stand, she would be offered termination of any pregnancy in which the fetus was male — the entire X chromosome serving as a crude and unreliable marker for the disease. The L1.28 marker makes it possible to distinguish fetuses bearing the normal X chromosome of the mother from those bearing the defective one — but only if the mother is heterozygous for the marker: that is, if she has A1 on one of her X chromosomes and A2 on the other. In that case, if her father's X chromosome bears the A1 variant she can be certain that any male fetus inheriting A1 will also inherit retinitis pigmentosa, and conversely if he inherits A2 he will be normal. But if she is homozygous — that is, she has A1 on both X chromosomes — there is no way of distinguishing a normal male from an affected one before birth. Given the frequency of the two variants in the general population, only 40 per cent of women will be heterozygous.

Similar considerations complicate the case of a woman for whom the family history of retinitis pigmentosa is on her mother's side. Because the symptoms of the disease in women develop late or never, she may not know even whether she is a carrier. If her mother is homozygous for the marker, the marker cannot enable her to find out; though it may, if she herself is heterozygous, enable the disease to be ruled out in some of her unborn sons.

Finally, even for a heterozygote, there is always the slight chance (decreasing with the closeness of the linkage) of misdiagnosis if the defective gene has become separated from the linked marker by genetic recombination. The identification of a second linked marker, on the other side of the gene, would dramatically reduce the probability of mistaken diagnosis; it would also be helpful in the pursuit of the retinitis pigmentosa gene itself.

This, of course, is the longer-term aim of the research: in the end the only entirely reliable genetic probe is the one that identifies the mutant gene. Progress will demand patience and ingenuity. Patience because the distance between L1.28 and the retinitis pigmentosa gene is something on the order of 3 million bases; so to reach the immediate vicinity of the gene will require either a marathon chromosome walk or laborious family studies to find more closely linked markers; and ingenuity to identify the gene once it has been isolated. For retinitis pigmentosa, the best bet may be to compare the genes expressed in normal retinas with those expressed in affected retinas, by means of cDNA libraries from the two tissues.

How soon might a gene probe for X-linked retinitis pigmentosa be available? Should female carriers or suspected carriers wait a year or two before starting families in the hope of reliable prenatal diagnosis? In the case of Duchenne muscular dystrophy, there is still no gene probe two years after the first marker; but optimists expect one this year. □

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Archaeology

The decline of Rome to a fast-food empire

from Richard Hodges

A NUMBER of recent archaeological publications provide evidence of a socio-economic transition that occurred in the Roman Empire as it declined and fell — 'the greatest, perhaps the most awful scene in the history of mankind', according to Edward Gibbon[1].

Whereas Gibbon's analysis of the faltering empire did not include such a transition, it had been anticipated by Henri Pirenne in his famous book *Mohammed and Charlemagne*, published half a century ago, the theme of which was returned to last year by C. R. Whittaker[2] who argued the revolutionary hypothesis that increased state exactions in kind in the later empire, and the effective decommercialization of the economy, reflected a reorganization of the means of exchange rather than economic decline. There was no fall, he contends, in production or exchange, only a decline in the entrepreneurial commodity market[3]. This reorganization, he argues, forms the foundation on which the medieval economy was constructed. But with such patchy documentation to support his argument, Whittaker, like Pirenne before him, is unable to measure change in production or distribution with any precision.

Archaeological evidence alone will adjudicate on this issue[4], and a recent volume of archaeological surveys in the Mediterranean area[5] pointedly illustrates one aspect of Whittaker's thesis. There was undeniably a change in the organization of production in the countryside towards the end of the fourth century AD; far fewer sites are characterized by the highly distinctive African Red Slip wares of the fifth and sixth centuries in all parts of the Mediterranean basin. Sadly, though, little attention is drawn to this fact — an occasion where archaeological statistics appear to be meaningful[6]. But what exactly does this reorganized rural pattern mean?

Alistair Small has excavated a Roman villa at San Giovanni di Ruoti in Basilicata that appears to be typical of the later Roman settlement system in Italy[7]. By early Roman standards it is a modest affair, but Small contends it is a normal residence of a *dominus*, a landlord in the fifth to sixth century. Of particular interest are the faunal and ceramic assemblages from the middens around the villa. As many as 40 per cent of the bones in the early fifth-century midden and 65 per cent in the later fifth-century midden are from pigs. The traditional balanced blend of meat consumption and stock-rearing on rural estates seems to have been abandoned in favour of the short-term fast production of pork. A passion for chops became the fashion of the time. However, while the animal bones reveal a trend towards fast-food processing, the pottery assemblage confirms the persistence, albeit in a re-organized form, of regional production–distribution services for ceramics. Several of the contributors to the volume in which Small's essay appears note the distinctive character of the later Roman economy as the state's role begins to diminish at a regional level.

Related information comes from the first part of David Whitehouse's important excavations at the foot of the Palatine[8]. From a study of the animal bones found there, Graeme Barker concludes that about 40 per cent of the meat eaten at this site was pork — a finding which he argues may be illuminated by the Theodosian code (AD 438) which describes guilds of pork suppliers who brought pigs from all parts of Italy for consumption by the poor of Rome. Pork, it seems, was the dole money of the Imperial City. Yet, as at S. Giovanni, the pottery assemblage at the *Schola* Praeconum indicates the existence of a vibrant market system. Large numbers of east Mediterranean amphorae and North African tablewares from the middens demonstrate far-flung mercantile connections of growing importance. So how does this evidence square up with Whittaker's thesis?

Two points emerge from the recent archaeological reports. First, short-term fast-food production and a trend towards more regionally devolved ceramic industries seem to have accompanied the drop in density of rural sites. Second, there were changes in distribution. Tax in kind, to judge from the pattern of pork consumption, was as significant as Whittaker anticipated. However, in contrast to the regionally discrete patterns of ceramic distribution evident in southern Italy, a marked increase in the volume of imported pots is evident in cities such as Rome[9]. The major cities, in other words, were booming and attracting the rural poor, the recipients of the pork dole, leaving *agri deserti* in the provinces, as Whittaker notes. Rome's Indian Summer, like that of the wealthier villas such as S. Giovanni, was notable for its emphasis on short-term consumption.

Whether the volume of production in this reorganized community remained static, as Whittaker contends, or declined, as seems more probable, will only be clear when the many regional archaeological surveys of the moment in Italy are completed. But all the current work demonstrates that these fifth-century developments reflect that time was running out for the Roman Empire. The state system began to gasp and collapse in the early sixth century before the Byzantine and then the Lombard invasions of the peninsula. The system of ports and villas fell apart soon after AD 500, leaving ruins which fascinated the early medieval elite as they do tourists today. Whittaker's thesis and his fascinating reworking of Gibbon's drama is to some extent confirmed by the archaeology of the period. The scale of change and the eventual collapse must have seemed every bit as awful to those who experienced it as Gibbon's gothic description contends. □

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Remains of a 5th–7th century later Roman villa at Anguillara, Lazio, Italy excavated by David Whitehouse for the British School at Rome. Photograph by courtesy of David Whitehouse.

Oceanology

Storms in the abyss

from P.F. Friend

ANY lingering notion that the dark waters of the deep ocean floors are uniformly calm should finally be dispelled by the vivid account of this remarkable environment provided by Hollister and McCave starting on pages 220 of this issue of *Nature*.

Their study concentrates on a 2 x 4 km area of the Atlantic Ocean, where it reaches a depth of almost 5 km, some 500 km south of Halifax, Nova Scotia. Although the surface waters there move generally eastwards, as part of the Gulf Stream, the deepest waters tend to move westwards as part of a distinctive deeper western boundary current that is cold (less than 1.9°C) and perhaps has its origins in the bottom water of the Antarctic. This cold bottom current has a remarkably high velocity of up to 0.73 m s^{-1}, similar to those measured in strongly moving surface tidal flows or in flooding rivers.

Another striking feature of the area is that it is subject to 'storm' periods of several days to a few weeks in which bottom currents intermittently reach speeds of over 0.3 m s^{-1} and are often reversed. Calculations of the variance of current speed provide a measure of the so-called eddy kinetic energy of the system. It seems that high values of the eddy kinetic energy in deep waters are usually found in areas where the eddy kinetic energy at the surface is also high, as in the Gulf Stream. The kinetic behaviour of the deep currents in storms is perhaps best visualized as an intermittent and reversing surge; there is no sign of the oscillatory periods of several seconds that are typical of surface-storm kinetics.

Because the muddy ocean floor in the area studied is rich in organic nutrients, it supports many actively burrowing organisms that soon disrupt any sedimentary features. The fact that the top layer of the floor retains sedimentary features therefore indicates that it was recently and rapidly deposited. The concentration of sediment can be as high as 12 g m^{-3} in these deep-bottom waters, similar to that in some muddy estuaries. These concentrations, coupled with the major variations in the small structures of the ocean bottom recorded in time-series of photographs, show that the vigorous movements of the bottom currents erode and transport large amounts of the fine-grained sediment. Indeed, careful examination of sediment cores from the ocean bed reveals the rapid rates of deposition of sediment and the occurrence of frequent episodes of erosion: several centimetres of mud may be deposited in the course of a few days and then removed equally rapidly. By such a process more than 3,000 times more material may have been deposited in the 10,000 years of the Holocene than is apparent from the deposition rate of 5 cm kyr^{-1} recorded in the sediments, according to the estimates of Hollister and McCave. It is common to find that the rates of short-term sedimentation (and erosion) are several orders of magnitude higher than those of long-term accumulation for the shallow-sea deposits in many old sedimentary basins but it has not hitherto been known that the same is true on the floors of deep oceans.

Considering all the deep oceans of the Earth, Hollister and McCave point to seven discrete areas, most of them tens to hundreds of kilometres across, where the suspended load of the deep-ocean water is particularly high. All the areas occur below zones of high surface-eddy kinetic energy, and probably contain deep currents which move strongly and reverse intermittently. These few, rather small areas may be the prime sources of much of the suspended muddy material of the world's oceans just as a few land areas, which have erodible soils and intermittently strong winds, seem to contribute a large part of the very fine-grained suspended terrigenous materials in the Earth's atmosphere. □

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Colour vision

Direct recordings from the cone cells of a primate

from J. D. Mollon

FACED with the infinite varieties of hues and tints in a sunlit wood in spring, it can be difficult to believe that all our sensations of colour arise from just three unidimensional retinal signals. Nevertheless, our ability to see in colour depends on no more than a comparison of the relative rates of absorption of photons in the three types of cone cell within each local region of the retina and on the distribution of these ratios across the retina. The three types of cone cell contain photopigments with peak sensitivity in different parts of the visible spectrum; although direct measurements of their absorbance spectra have been possible for many years, Nunn, Schnapf and Baylor have achieved a great technical feat in obtaining the first direct electrophysiological recordings from a primate cone cell, reported on page 264 of this issue[1].

The existence of three types of retinal receptor was first suggested 200 years ago[2,3] and by 1886 the brilliant Arthur König had accurately deduced the spectral sensitivities of the cones from psychophysical studies of normal and colour-blind observers[4]. But the three types of cone remained, in effect, hypothetical constructs until the first direct measurements of the absorbance spectra of the different cone types were obtained by microspectrophotometry, in which a narrow monochromatic measuring beam is passed through the outer segment of an individual receptor cell. Nowadays relatively precise estimates of the three absorbance spectra are available for monkey and human retinas[5,6].

An absorbance spectrum — a plot of the optical density of the cell at different wavelengths — is not, however, necessarily the same as an electrophysiological action spectrum, which shows the number of photons required at different wavelengths to produce a given electrical signal. For example, it might have been the case that the electrical signal produced by an absorbed photon was dependent on wavelength. Moreover, the steep long-wavelength limbs of the absorbance spectra can be followed only down to values around one-tenth of the maximum absorbance, whereas action spectra are not so limited and it is easy to measure the intense lights needed to produce a criterion electrical signal at long wavelengths.

It is physiological action spectra that Nunn, Schnapf and Baylor have now provided us with. To measure them in the tiny (perhaps 2μm by 10μm) pigment-containing outer segments of primate cones, they adapted a clever trick that Yau, Lamb and Baylor[7] introduced in 1977 originally to make recordings from the much larger photoreceptors of the toad. Instead of trying to put a microelectrode inside the outer segment, Yau *et al.* put the outer segment inside a micropipette, sucking it gently in and allowing the mouth of the pipette to form an electrical seal round the base of the outer segment. The signal thereafter measured is a graded reduction in the current that flows continuously into the outer segment in the dark.

In their samples of tissue from macaque monkeys, Nunn *et al.* have been able to record only middle- and long-wavelength cones; the peak sensitivities of the two types of record lie quite close together in the green (540 nm) and yellow-green (570 nm) regions of the spectrum, in good correspondence with both psychophysical

and microspectrophotometric estimates. The short-wave receptors, the so-called 'blue cones', have so far eluded measurement by the new method. But this is not surprising, for psychophysical, microspectrophotometric and histological results all converge to suggest that the blue cones are relatively sparse, perhaps accounting for less than 10 per cent of all cones in human and macaque retinas. It seems that we use the blue cones only for colour vision *per se*. For the detection and resolution of contours, for stereopsis and for discrimination of movement, we rely on visual subsystems that draw their inputs only from the long and middle-wave cones[8].

Of particular interest to many psychophysicists will be Nunn *et al.*'s observation that the electrical responses of primate cones have a shorter time course than do those of rods from the same species. Such a difference has long been postulated in order to explain why our ability to resolve rapid flicker is poorer, and why the subjective duration of our sensations is greater, when our vision depends on responses of the rods rather than of the cones[9].

Building on this pioneering work of Nunn, Schnapf and Baylor, it will be possible to answer some interesting questions. First, there is the question of adaptation. Nunn and Baylor have already reported that primate rods exhibit little intrinsic gain control as the illumination level is increased[10]. Will this also be the case for primate cones? Second, will there prove to be a residual discrepancy between absorbance and action spectra for primate cones and, in particular, is the quantum efficiency the same for the short-wave 'β band' as for the primary mode of the spectral sensitivity curve? These questions will be securely answered only when microspectrophotometric and electrophysiological measurements are made concurrently on the same primate cones. Third, will there prove to be individual differences in the exact spectral positions of cone sensitivities as has been suggested by microspectrophotometric data from human eyes[6]? □

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Molecular biology

Anatomy of hypersensitive sites

from Sarah C. R. Elgin

In the analysis of the structure of chromatin in animal cells, sites hypersensitive to digestion by the enzyme DNase I are generally found within the 1,000 base pairs (bp) that flank the 5′ ends of genes that are active or can be activated (see ref. 1 for a brief review). That these discontinuities in the chromatin fibre mark sites relevant to the control of gene expression has been shown in several instances, most directly in an analysis of deletion mutations at the *Drosophila Sgs4* glue protein locus[2], and they can be considered as sites of potential interaction between specific effector proteins and DNA. A critical question is that of how the DNase I hypersensitive sites are generated and maintained; answers should not be long delayed to judge by the progress that has already been reported this year or is soon to be published.

The appearance of DNase I hypersensitive sites precedes the initiation of transcription and is tissue-specific for genes so regulated. Studies of the minichromosome of simian virus 40 (SV40) and of the chick β-globin gene indicate that DNase I hypersensitive sites correspond to regions of DNA that are nucleosome free[3-5]. Since it is known that DNA in certain conformations is unlikely to form stable nucleosomes, a formal possibility is that the DNase I hypersensitive sites consist solely of a short stretch of DNA in one such conformation. In that respect there has been considerable recent interest in sites that are sensitive to S_1 and other 'single-strand-specific' endonucleases in regions 5′ to genes in supercoiled DNA and in chromatin[6,7]. Some are sensitive to S_1 nuclease but not *Bal*31 nuclease, while others show the opposite pattern[11]. The sites frequently correspond to short stretches of double-stranded DNA where one strand is all purines and the other all pyrimidines[8-10]. The exact form of the altered structure at these sites in the supercoiled DNA is unknown but it appears unlikely that truly single-stranded regions are formed[10,11].

While the available data are sketchy, it is common to observe, 5′ to genes, one of several classes of sequence capable of adopting some sort of altered conformation. Comparisons of DNA mapping data with chromatin mapping data indicate that in at least some cases, such sequences flank DNase I hypersensitive sites of chromatin instead of being an integral part of them (for example, refs 11,12). This is not to suggest that they are necessarily without influence; data both from artificial rearrangements of the SV40 genome and from *Drosophila* variants at the *Sgs4* locus indicate that sequences adjacent, or within a few hundred base pairs, can be critical for the formation of DNase I hypersensitive sites[2,13]. A model in which alternative DNA structures play a part in generating DNase I hypersensitive sites is attractive[6,7]. One's enthusiasm must, however, be tempered by the fact that there is relatively little evidence of whether alternative forms of DNA actually occur *in vivo*.

It seems likely that DNase I hypersensitive sites are generated by the interaction of nonhistone proteins with the DNA, perhaps following cues from the surrounding sequence, perhaps binding to the region itself. The challenge to isolate such proteins has recently been taken up by Emerson and Felsenfeld[14]. Using as an assay the correct assembly of the DNase I site 5′ to the chick $^{A}\beta$-globin gene, they have separated from the nuclei of chick erythrocytes, in which the gene is expressed, a protein that not only produces the requisite nuclease sensitivity but also retains a DNA fragment containing the 5′-flanking region in a filter-binding assay. Binding experiments with different DNA fragments set one boundary of the recognition site between 268 and 303 bp, and the other between 40 and 164 bp, 5′ of the transcription start site. For comparison, the DNase I hypersensitive site maps between 65 and 163 bp, micrococcal nuclease sensitivity extends to 260 bp (ref. 5) and a tract of 16 consecutive deoxyguanosine residues, sensitive to S_1 nuclease in the supercoiled form, occupies bp 178–197 (refs 9 and 10). The region of interest therefore covers both the DNase I hypersensitive site in chromatin and the most prominent S_1 nuclease sensitive site in the DNA, but not the 'TATA box' or the transcription start site. Obviously more than one critical protein–DNA interaction may be occurring.

Fortunately, several new techniques have recently been developed which improve our ability to map or 'footprint' both alterations in DNA structure and protein–DNA interactions. In a forthcoming issue of *Nature*, M.M. Becker and J.C. Wang will describe a 'photofootprinting' technique, which uses ultraviolet light to detect protein–DNA contacts as well as changes in the structure of DNA at the sequence level. The technique is based on the observation that the photochemical reactivities of DNA bases are dependent on both protein-binding and DNA structure. Breakage of the DNA backbone only at the sites of photodamage and end-labelling by any of several techniques allows a map of relative photoreactivity to be developed. The question of which conformations of DNA at 5′ sites with potential alternative structures are present *in vivo* might profitably be addressed by this method in

the immediate future.

And on page 229 of this issue, Wu introduces a new approach to analysing the position of DNA-binding proteins within or around hypersensitive regions[15]. He uses exonuclease III of *Escherichia coli* to digest chromatin within the hypersensitive site after its cleavage by a suitable endonuclease (DNase I or a restriction enzyme). The exonuclease is thought to stop when it encounters a protein, thus defining one boundary of the binding site. In an analysis of a heat-shock protein gene (*hsp70*) of *Drosophila*, a resistant (protein-bound) site is found 12–40 bp 5′ of the transcription start site in normal cells; in heat-shocked cells, however, an additional resistant site is observed in the region between 40 and 108 bp.

Techniques recently devised to cross-link protein with DNA should provide a second method for mapping these interactions, and the potential to identify the proteins involved, although at present this is frequently limited to cases where antibodies against the protein are available (for example, refs 16,17). Concerted application of these techniques could lead to a full definition of the DNase I hypersensitive site in molecular terms within the next few years. □

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Archaeological dating

New method for bricks?

from E.T. Hall

AN unexpected byproduct of investigations of the chemical stability of clays employed in the storage of nuclear waste is the suggestion of a new method of dating bricks, ceramics and other materials fired in antiquity. The technique, suggested by Waddell and Fountain[1], depends on the diffusion of Ca^{2+} ions from mortar or cement layers into the clay brick. The authors report a relationship between time and calcium content at the interface of the clay brick with the mortar but base their report on only seven samples. These show that there is an increase of calcium with time, although the experimental nature of the curve relating the distance migrated by calcium to time is far from clear. The migration distances are small (< 20 μm in 200 years), whereas the matrix of bricks is comparatively coarse. An electron microprobe was used for the analyses, providing a resolution of 2.5 μm; there can be little doubt that the analytical results from the traverses across the cement–brick interface must be very 'noisy' and accurate measurement difficult to achieve, even though multiple passes were made to obtain an average result.

The new technique is interesting but still at a preliminary stage of development and its application to real archaeological dating may well be problematical. Clearly it has a long way to go before it can rival the most successful of the methods for dating fired materials — thermoluminescence. The work of Aitken[2,3] and others[4] has made thermoluminescence the standard procedure[5] for testing the authenticity of doubtful ceramics, particularly when public or private purchasers are contemplating their purchase in the saleroom. For bricks whose environment is known, so that the thermoluminescent component arising from their surroundings or from cosmic rays can be estimated, thermoluminescence can be expected to provide dating that is accurate to within ~7 per cent.

Another technique for dating bricks and other fired clays is archaeomagnetic dating[6], where the past history of the Earth's magnetic field, both in direction and intensity, is used. In the case of bricks, which will have the direction of the Earth's field induced in them at the time of firing, we do not normally know the orientation of the brick when fired and so measurement of direction of the brick's magnetic moment will not help us. However, recent measurements[7] of intensities of magnetization, which can be related to the ancient field strength, have provided some evidence that this might become a useful method of dating, albeit at a lower accuracy than is provided by thermoluminescence. □

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Fractals

Aggregationism

THE problem of aggregation is both ancient, as in the manufacture of carbon black, and modern, with the recognition in the past few years that microscopic structures as different as snowflakes and colloidal precipitates may be examples of physical structures with fractional dimensions — fractals in the sense intended by Hausdorff in 1919 and more recently given wide currency by the proselytizing of B.B. Mandelbrot. One page 225 of this issue R.M. Brady and R.C. Ball report one of the most direct demonstrations so far that aggregation does yield fractal structures.

Brady and Ball are concerned only with aggregation whose rate is limited by that at which extra particles can reach the surface of a growing cluster. The circumstances therefore do not necessarily correspond to those which determine the growth of, say, a snowflake from water vapour, where the shape of the crystal may be governed by the rate at which latent heat can escape. Either way, the application of macroscopic arguments to the growth of these structures is hampered by the non-linear character of the instabilities that form on growing surfaces (departures from regular shape grow more quickly) whence the interest in the microscopic simulation introduced by T.A. Witten and L.M. Sander three years ago (*Phys. Rev. Lett.* **47**, 1400; 1981).

That model is simple enough. Put a seed particle at the centre of a finite patch of, say, a square lattice and allow fresh particles to enter the system at random places on the periphery and to make their way through the lattice by random walking, following the rule that they stick to the growing aggregate whenever they reach a neighbouring position on the lattice. Brady and Ball confirm the computer prediction for the three-dimensional lattice (that the density should be a decreasing function of the radius of an aggregate) by their neat set-up in which the electrolysis current at constant voltage is simultaneously both a measure of distance and, when integrated over time, a measure of the total mass.

As it happens, an experimental investigation of the other obvious case of aggregation — that typified by the aggregation of colloidal particles in homogeneous suspension — has also just been published (Weitz, D.A. and Oliveria, M. *Phys. Rev. Lett.* **52**, 1433; 1984). The model, in this case, is one in which the particles initially occupy many sites of a lattice, then diffuse randomly through it and stick together whenever they encounter another particle or growing aggregate. The unpublished result of the simulation (due to P. Meakin) is said to be a fractal dimension of about 1.75, which Weitz and Oliveria say they have confirmed by the measurement of individual particles of colloidal gold in aggregates formed by controlled flocculation. □

Role of RecA protein spiral filaments in genetic recombination

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Physical and enzymatic studies on RecA protein from Escherichia coli *provide the basis for a molecular model of general genetic recombination, a novel feature of which is the role attributed to spiral filaments of RecA protein.*

IN general genetic recombination, homologous chromosomes are paired, cut and joined to form chromosomes with new linkages. To examine the underlying mechanisms, data from genetic crosses in fungi, bacteria and viruses have been used to develop models that account for the genetic data. More recent biochemical and physical studies on RecA protein, the recombination enzyme of *Escherichia coli*, allow the development of more complete molecular models for the pairing and exchange reactions involved. In this article we describe such a molecular model, introducing it with a brief review of published models and the studies on which it is based.

Models of genetic recombination

Several alternatives have been suggested to explain how homologous pairing between chromosomes is initiated: (1) when both duplexes are nicked by an enzyme at identical positions in each chromosome[1]; (2) when one duplex carries a single strand gap and the other is nicked[2,3] (Fig. 1*a*); (3) when one duplex is nicked and a strand is displaced by local DNA synthesis[4] (Fig. 1*b*); (4) when both duplexes are replicated locally[5]; (5) when particular sites in DNA are recognized by initiation enzymes[6]; (6) when a two-strand break is made (Fig. 1*c*)[7]; or (7) when one duplex carries a single-strand gap and the other is intact[8–11] (Fig. 1*d*). The more recent models were designed to form unequal lengths of heteroduplex DNA, as required by the data on meiotic recombination in fungi[7,12,13]. However, it has proved difficult to determine from genetic data how recombination is initiated, and the choice between models remains open.

Homologous recombination also serves a function in the repair of certain types of DNA damage. In yeast, for example, the repair of double-strand breaks depends on recombination with a homologue[14]. Similarly, the switch between mating types is attributed to gene conversion at the mating type locus, initiated by a double-strand break[15]. In bacteria, recombination is initiated by DNA containing incised cross-links[16] or by post-replication gaps which induce sister exchanges with an efficiency close to unity[8].

In *E. coli*, both general genetic recombination and post-replication repair require *recA*$^+$, a pleiotropic gene that is activated by DNA damage[17,18]. The *recA*$^+$ gene product[19–23] is a protein of 38,000 molecular weight (M_r) and known sequence[24,25] that polymerizes into spiral filaments. It has a single-strand DNA-dependent protease activity for SOS induction in response to DNA damage[23], and it mediates enzymatic reactions with DNA of relevance to recombination[3].

DNA binding sites

In the presence of the non-hydrolysable ATP analogue, ATP(γ-S), RecA protein tends to aggregate at low *p*H into long filaments[26], and at neutral *p*H it condenses on duplex DNA in rod-like filaments[27]. On careful examination of shadowed specimens in an electron microscope, the filaments formed on DNA are seen to be striated and to consist of right-handed spirals of pitch 100 Å and diameter roughly 100 Å (Fig. 2*a*, *b*). When negatively stained, the spiral filaments appear to be deeply grooved with space between adjacent turns. They occur singly or in bundles in quasicrystalline alignment and appear hexagonal in cross-section[28].

The number of turns of spiral filament needed to cover a circular plasmid of defined length is very reproducible, indicating that RecA binds stoichiometrically to duplex DNA. There is one turn per 18.6 base pairs (bp) and there are 6.2 RecA monomers per turn, as determined by counting RecA striations on plasmid DNA and by measurements of the linear density of the filaments[29,30]. Evidently the spiral protein filament is wound along the phosphodiester backbone of the duplex with each monomer binding to exactly the same number of base pairs[28,29,31]. This number may be 3 bp, as assumed in Fig. 4, but not all methods give the same value[32]. Measurements of overall length of these filaments show the DNA to be stretched by 50% beyond the normal B-DNA length. At 18.6, instead of the usual 10 bp per turn, the duplex is underwound by nearly 50%, the angular twist between adjacent base pairs being reduced from the normal value of 36° to about 20° (refs 28, 29).

RecA protein filaments also form on circular single-strand DNA in the presence of ATP, but are neither stretched nor striated. Some complexes are seen by electron microscopy to be of similar length to B-DNA, but others are collapsed on themselves. They are unstable and few are seen unless single-strand binding protein is present, or they are fixed with glutaraldehyde[33].

Certain features of RecA spiral filaments have been revealed by X-ray crystallographic methods. RecA protein crystallizes in hexagonal and in tetragonal crystals, which have been examined by X-ray diffraction[34]. The hexagonal crystals which form at low *p*H appear to contain spiral chains with 6 RecA protein monomers per turn of length 84 Å and symmetry P6.1 (Fig. 2*c*). Since the spiral filaments seen by microscopy are right handed, we think the monomers may also be arranged in right-handed spirals within the crystals. The X-ray diffraction studies on hexagonal crystals prove that the spiral filaments consist of RecA monomers arranged head to tail in equivalent positions (Fig. 2*c*), and not in a sequence of dimers or tetramers[34]. The head-to-tail orientation of monomers provides a physical basis for the functional directionality of the enzyme in strand exchange reactions[35–37]. This directionality can be understood if each monomer has two DNA binding sites, one for each DNA molecule to be paired. In contrast there would be no basis for directionality if each monomer had only one DNA binding site and the functional unit was a symmetrical dimer.

In summary, these physical methods show that RecA protein binds to DNA stoichiometrically and forms right-handed spiral

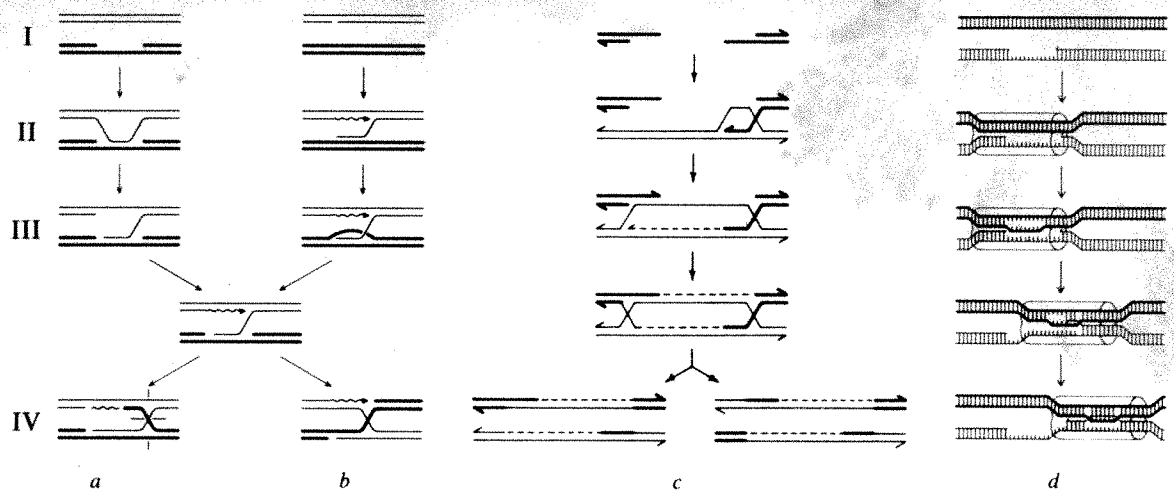


Fig. 1 Current models for the mechanism of general genetic recombination. *a*, (I) Recombination initiated by a single-stranded gap. (II) Unwinding and pairing. (III) Paired strand is cut. (IV) Repair synthesis. (V) Isomerization and resolution of one crossover to form recombinants[2,3,10]. *b*, (I) Recombination initiated by a nick. (II) DNA synthesis at nick displaces strand. (III) Homologous pairing. (IV) Intact duplex is cut in response to pairing. (V) Isomerization and resolution of one crossover to form recombinants[4]. *c*, (I) Recombination initiated by a two-strand break in one duplex. (II) pairing by first 3′ end. (III) Repair synthesis from 3′ end, second 3′ end pairs. (IV) Repair synthesis from second 3′ end completes gap repair and formation of two cross-overs. (V), (VI) Resolution of two cross-overs to form recombinants[7]. *d*, (I) Recombination initiated by a single-strand gap. (II) RecA protein condenses on gap and promotes homologous pairing. (III) Strand exchange to form heteroduplex at gap. (IV) Branch migration to the right allows end of strand to interwind with duplex. (V) Strand crosses back. The gap is repaired and the two cross-overs are resolved to form recombinants. The intact homologue is cut only at the resolution of the cross-overs[11].

filaments of regular structure with all the monomers orientated in the same direction.

Initiation, pairing and strand exchange

RecA protein binds cooperatively to single-strand DNA and has a single-strand DNA-dependent ATPase activity[38,39]. Homologous pairing by RecA protein was first detected *in vitro* when single-strand fragments were paired with circular duplex DNA to form stable complexes[40–43] referred to as displacement loops or D-loops. However, the pairing and strand exchange reactions of this protein have been more fully characterized in reactions with circular single-strand and full length linear duplex DNA[44,45]. RecA protein binds to single-strand DNA to form an initiation complex that is capable of immediate pairing with duplex DNA[46,47]. One strand of the linear duplex is transferred to the single-strand circle, to form a nicked circular heteroduplex and a linear single strand[44]. The strand exchange requires ATP and advances in the 5′ to 3′ direction along the original single strand at a rate of only a few bases per second[35,36]. It works best if single-strand binding protein (SSB) is added to stabilize the initiation complex, and has the advantage that the substrates, intermediates and products of the reaction can all be assayed.

Homologous pairing and strand exchange reactions also occur between two duplex molecules if one of them contains a small single-stranded gap (gapped DNA)[9,10]. When gapped circular DNA is incubated with RecA protein and linear duplex DNA containing a 3′ end complementary to the gap, RecA pairs the molecules starting in the gap[48,49]. Strand exchange then proceeds in the 5′ to 3′ direction in the gap and continues along the adjoining duplex to form two heteroduplexes[11,37]. When the gapped DNA is incubated with nicked circular DNA, pairing at the gap leads to the 3′ ends being exchanged and to the formation of figure-8 DNA structures containing Holliday cross-overs[50].

The reaction between gapped and intact circular duplex DNA is similar. Again, homologous pairing initiated at the single-strand gap, leads to strand exchange and the formation of heteroduplex DNA, but in this situation the length of heterodu-

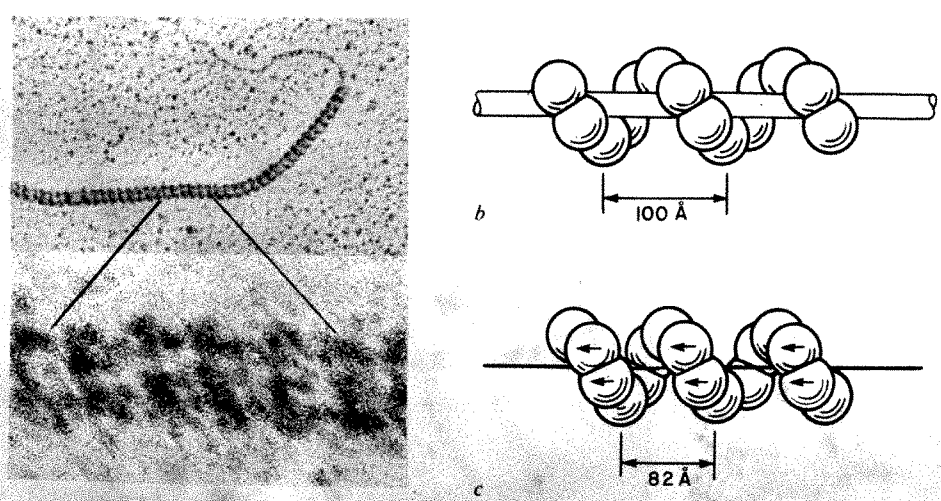


Fig. 2 Structures of RecA protein spiral filaments. *a*, RecA protein spiral filament formed on circular duplex DNA in the presence of ATP(γ-S), shadowed with Pt and seen by electron microscopy[28]. *b*, Diagram of RecA bound to duplex DNA in the presence of ATP(γ-S), as determined by electron microscopy[28]. RecA monomers are shown as spheres, but their exact shape is unknown. *c*, Diagram of RecA spiral filament in crystals of RecA protein free of DNA, based on X-ray crystallographic data. Arrows indicate alignment of monomers[34].

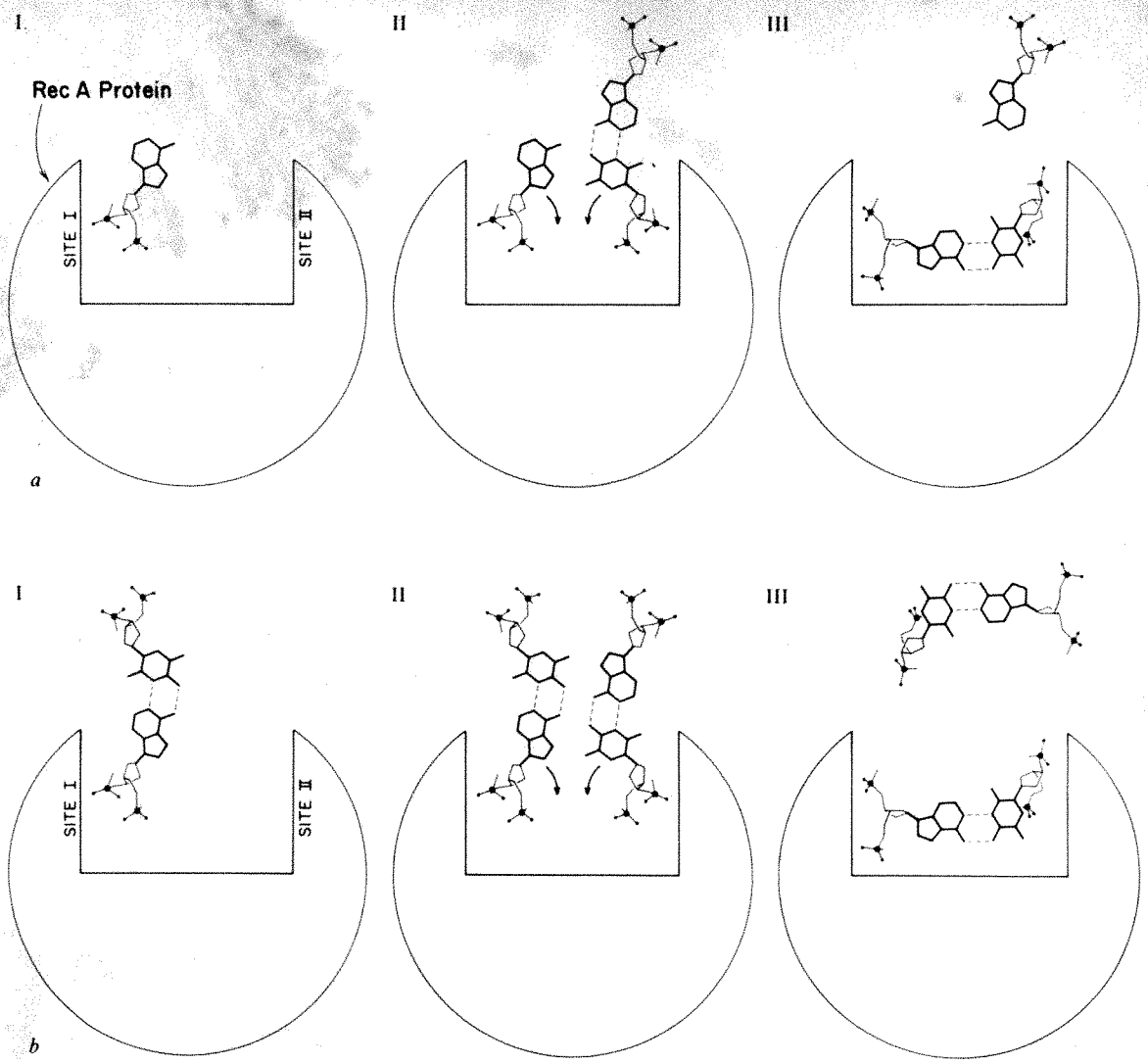


Fig. 3 A two DNA binding site model for RecA protein and a proposed mechanism for homologous pairing and strand exchange. Each base is shown in axial view with two phosphates. The sugar phosphates are unwound to about 20° between adjacent base pairs. RecA protein is about 50 Å in diameter, 60% larger than shown in this diagram. *a*, Pairing and strand exchange between single strand and duplex. (I) A single-strand binds at site I of RecA protein to form an initiation complex. (II) Initiation complex binds to one strand of a duplex at site II with homologous pairing by hydrogen bonding (bonds not shown) in the wide groove. (III) RecA protein rotates the bases into the heteroduplex position. *b*, Pairing and strand exchange between two duplexes. (I) One strand of the duplex binds at site I to form a duplex initiation complex. (II) The duplex initiation complex binds to the second duplex with homologous pairing by hydrogen bonding (bonds not shown) in the wide groove. (III) RecA protein rotates the bases into the heteroduplex configuration.

plex is limited by topological constraints and an interwound figure-8 carrying two Holliday cross-overs is formed[10,11].

Search for homologous contacts

Several mechanisms for homologous pairing can be visualized: (1) RecA protein may move two DNA molecules relative to each other until homologous sequences come into alignment, or (2) pairing may result from random contacts between DNA molecules. In support of the latter mechanism, RecA pairs single-stranded and duplex molecules if they share a common base sequence of 100 bp or more, but fails to do so if homology is limited to 30 bp. The rate of homologous pairing is directly proportional to the length of the DNA molecule, even if partially non-homologous[51]. Moreover, RecA protein forms aggregates with DNA, that are readily seen by electron microscopy and must locally increase the concentration of both naked and complexed DNA[27,33]. These results are consistent with a pairing mechanism in which DNA, aggregated by RecA protein, makes an increased number of random contacts at multiple points along chains. Such contacts will of course be transient unless homologous sequences touch and pairing is established.

The alignment of identical base sequences at homologous pairing presumably depends upon sequence specific complementary hydrogen bonding between DNA chains. Two forms can be visualized: (1) The two strands of a duplex may be separated and able to pair as Watson–Crick heteroduplexes. This form of pairing may occur in the absence of RecA when superhelical circular phage DNA is annealed with homologous single-strand fragments to form D-loops[52]. However, it is not at all clear that this form of pairing occurs in RecA-mediated reactions, particularly since *in vitro* tests for RecA-mediated strand separation of duplex DNA in advance of pairing have given negative results[53–55]; (2) Initiation complexes may pair in the wide groove of the duplex. Complementary base pairing between a single-strand and a fully base-paired duplex could depend on hydrogen bonds between the oxygen and amino groups at positions 4 on pyrimidines and positions 5 and 7 on purines. A four-stranded structure that depends on such bonding has been proposed[56–58]. Poly(dT) · poly(dA) · poly(dT) is not of exactly the same structure, but is an example of a stable three-stranded deoxyribose polynucleotide chain[59].

Although we cannot rule out either form of pairing on the

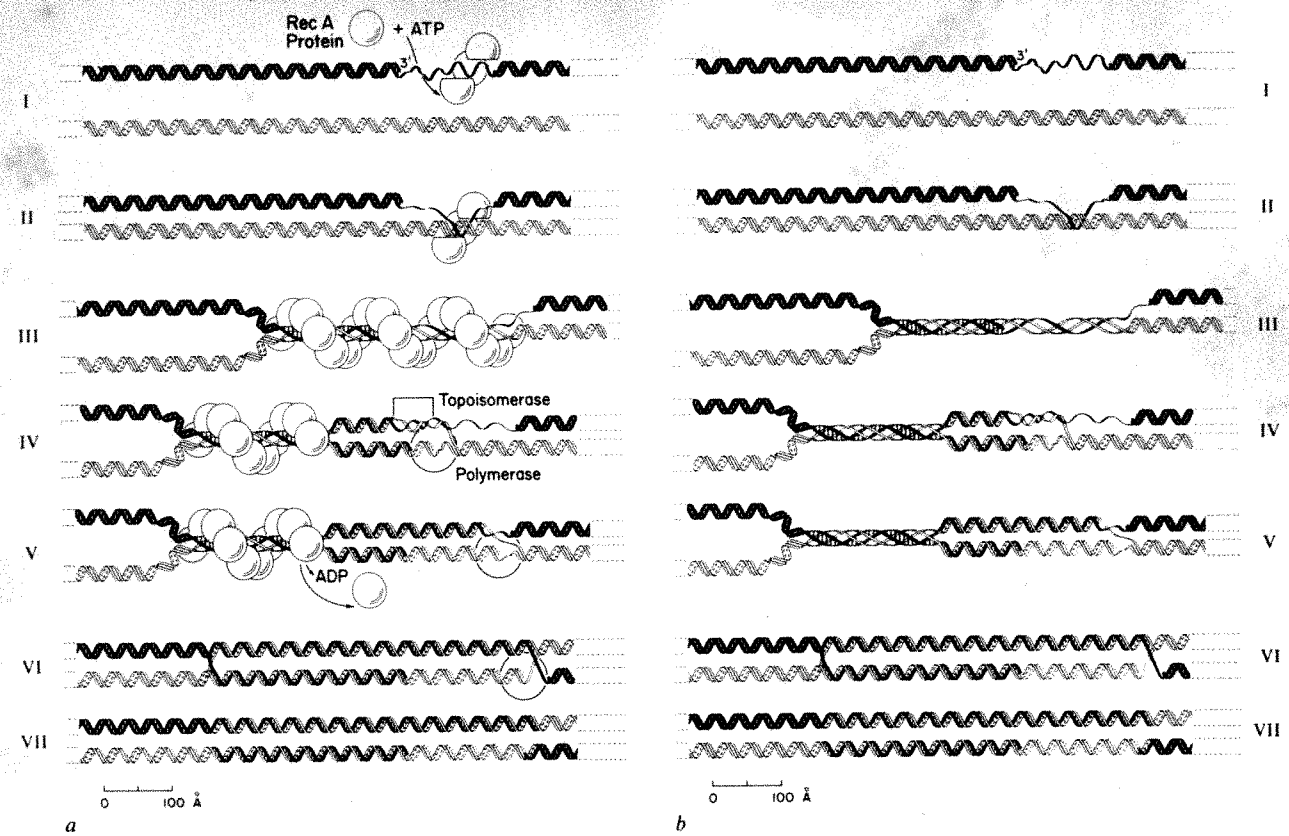


Fig. 4 An enzymatic model for genetic recombination. *a*, Complete model with enzymes; *b*, DNA only. (I) RecA protein binds cooperatively to the single strand in a gapped duplex to form an initiation complex, in preparation for pairing. Watson–Crick base pairs are shown as rods linking the DNA twin helices. (II) The initiation complex binds to the intact duplex, making transient contacts until a homologous site is reached. For clarity, the initiation complex is drawn with only a few protein monomers, but in reality is likely to extend over hundreds or thousands of nucleotides. (III) When homologous contacts are made and the strands become paired locally, the initiation complex acts as nucleus for further cooperative binding, which extends the RecA spiral filament around all three paired strands. As more RecA binds, the spiral filament extends around both duplexes drawing them together, wide groove facing wide groove. RecA binds to duplex molecules in response to pairing. (IV) The spiral filament is extended further by the addition of more monomers to the head end. RecA monomers at the tail end switch the strands to the heteroduplex configuration and may then be released from the DNA. The upper heteroduplex is relaxed by topoisomerase I. DNA polymerase starts to repair single strand gap by adding nucleotides to 3′ end (broken lines). (V) RecA driven branch migration continues with hydrolysis of ATP. (VI) Growth of the spiral filament ceases and the last RecA monomer dissociates, leaving single strand cross-overs, which are then resolved enzymatically. The crossover on right is generated by switching the base stacking (isomerization). (VII) Mismatched base pairs in the heteroduplexes are corrected enzymatically and mature recombinants are formed.

basis of experimental data, we think wide groove pairing to be more likely because of the failure to detect strand separation before pairing and because wide groove contact would hold the strands in position for the ensuing strand exchange reaction. Moreover, it is valid for pairing between single-stranded and duplex DNA as well as for the pairing between two duplexes that should occur in reactions between gapped and intact duplex DNA[9–11] (see also Fig. 3*a*(II), *b*(II)). Although stable four-stranded polydeoxyribonucleotide chains have not been found, they may be formed when two duplexes are held together within a spiral filament. However, the duplexes probably would not be in the normal B-form, nor in a fully paired four-stranded structure with 3.4 Å axial spacing as has been proposed[56,57]. Since RecA spiral filaments on duplex DNA are regular structures in which the DNA is underwound and stretched by 50% (refs 28, 29), the four-stranded structure is likely to be regular rather than a mixture of left- and right-handed turns, and to be symmetrical about the helical axis.

Surprisingly, strand exchange reactions do not require the substrate to be undamaged or even that the duplexes are fully homologous. For example, in a three-strand reaction, strand exchange is affected very little by deletions of 10 or 20 bp[60,61] or by pyrimidine dimers[62]. Similarly, deletions of a few base pairs in one duplex do not block strand exchanges between two duplexes[50]. Thus, in contrast to the initiation of homologous pairing which is sensitive to departures from homology, the strand exchange reaction advances along each duplex with little regard for small deletions or base damage.

RecA binding to duplex DNA

The binding of RecA protein to DNA molecules exhibits several unexpected features. In the presence of ATP, it normally binds only transiently to covalent circular duplexes. However, the addition of homologous single-strand DNA fragments results in a more stable form of binding of RecA to the double-stranded DNA, which then becomes temporarily immune to subsequent pairing with single-strand fragments[63–65]. This immunity may be due to the RecA protein which, starting from the paired initiation complex, binds cooperatively to the circular duplex and causes local underwinding. The remainder of the form I DNA becomes overwound and the whole molecule becomes immune to pairing. Further evidence for underwinding by RecA is obtained in similar reactions in the presence of topoisomerase I. Again the addition of homologous single strands leads to extensive RecA binding and to a marked reduction in DNA winding number[54,64].

In summary, RecA binds to ssDNA to form initiation complexes which are ready for pairing, but it binds to duplex DNA in response to homologous pairing, forming a complex which is immune. These results provide additional evidence that each RecA monomer has two DNA binding sites (Fig. 3*a*). Initiation complexes formed by interaction of the ssDNA fragment with

site I, pair with duplex DNA which binds at site II. Since the resulting ternary complex acts as a nucleus for further cooperative RecA binding beyond the terminus of the single stranded fragment, the duplex DNA becomes covered by RecA protein and is subsequently immune to further pairing.

A molecular model for recombination

Based on the information discussed above, we have constructed a molecular model for general genetic recombination in *E. coli*. It differs from previous models by proposing that RecA protein forms a spiral filament that surrounds the paired DNA molecules. The strand exchange reaction takes place within the spiral filament and switches base pairing to form two heteroduplexes. To illustrate this mechanism, we show a diagram of an exchange between two duplexes initiated at a single-strand gap, but anticipate that this mechanism may be applicable to recombination initiated in other ways.

The assumptions made in developing this model are as follows: (1) Each RecA monomer has two distinct DNA binding sites, the primary site and the secondary site (Fig. 3*a*(I) and (II)). (2) In an initiation complex, the primary binding site in each RecA monomer is filled by single-strand DNA. (3) Pairing is between an initiation complex and a naked duplex. (4) The RecA spiral filament winds round the paired DNA molecules and dissociates after strand exchange.

Figure 4a shows an outline of the proposed molecular model for recombination between two duplexes, and Fig. 4b shows the same model with enzymes removed, so the DNA strands can be seen more clearly. In this scheme, genetic recombination is initiated by the duplex containing a single-strand gap. Since RecA protein forms initiation complexes on single-strand DNA[46], we will assume it also does so on gapped DNA (Fig. 4*a*I), binding initially to the single-stranded DNA in the gap and subsequently along the adjoining duplex[27]. Once formed, initiation complexes pair with naked duplex DNA (Fig. 4*a*II). For clarity, the initiation complex is drawn in Fig. 4 with only a few protein monomers, but in reality is likely to be many times longer.

Homologous pairing between gapped and intact duplex molecules may occur when the initiation complex (Fig. 4*a*I) with single-stranded DNA at site I (Fig. 3*a*I), makes homologous contacts with a naked duplex, winding around all three strands (Fig. 4*a*II) with the duplex at site II (Fig. 3*a*II). Compensating left-hand turns which must form outside the paired regions, are not shown[58]. The paired initiation complex may serve as a nucleus for further cooperative binding, extending the spiral filament to the end of the gap and along the adjoining duplex, drawing both duplexes together (Fig. 4*a*III). In a similar way, when site I is filled by duplex DNA, it behaves as an initiation complex and is again capable of interacting with a naked duplex which binds in site II (Fig. 3*b*II). Thus, a single mechanism suffices for both three- and four-strand interactions. We will assume that the filament is orientated and grows by accretion at one end, which we will call the head end, and dissociates by loss from the tail end, the head to tail direction being set by the 3′ (head) to 5′ (tail) direction along the single strand. Once an initiation complex has established homologous contacts with a duplex, and the paired strands are surrounded by the spiral protein filament (Fig. 4*a*III), the strand exchange reaction may proceed. We suggest it may occur at the terminal monomer at the tail end and depend upon allosteric changes associated with ATP hydrolysis, in which the bases are rotated out of their original Watson–Crick pairing to form heteroduplexes as shown in Fig. 4*a*(III) and (IV) and more clearly in a plane at right angles to the helical axis in Fig. 3*a*(III) and *b*(III). In space-filling molecular models, the bases are easily rotated into the required positions by rotation mainly about phosphate-oxygen bonds.

Since each RecA protein binds to more than one nucleotide, it may switch several base pairs at a time. Figure 4*a*(IV) and (V) show the terminal monomer switching 3 bp and then dissociating, leaving the next monomer to perform the same reaction. Thus, we visualize that the spiral filament grows at the head end by monomers binding cooperatively, drawing the duplexes together, while monomers are released from the tail end as heteroduplex DNA is formed. The length of the spiral filament may therefore be variable, depending upon the balance of accretion at the head end dissociation from the tail end. The reaction will be completed when RecA protein, for whatever reason, fails to add further to the growing end, and the last monomer of the spiral filament dissociates.

Of the two heteroduplexes formed, the lower one should be free to coil into a relaxed duplex because of the single-strand gap, while the upper heteroduplex, having no gap, will be constrained but may be relaxed by topoisomerase I (Fig. 4*a*(IV)). The reaction is completed by a switch in base stacking referred to as isomerization[4] to provide the template needed for gap repair by DNA polymerase and ligase (Fig. 4*a*(V) and (VI)).

At this point, the gap has been repaired, the two duplexes are freed from the spiral filament, and they are joined by two single-strand cross-overs (Fig. 4*a*(VII)). They are converted to mature recombinants by the enzymatic resolution of cross-overs and the repair of mismatches in the heteroduplexes[50]. The endonuclease activities performing these functions are still to be purified and studied.

Future prospects

Several features of the proposed enzymatic model require further investigation. The basic structure of homologous pairing has not been established. RecA binding to DNA and the structure of the resulting complex require further examination. Conflicting results on the stoichiometry of RecA binding need to be resolved. We do not know what triggers a strand exchange or how it takes place. We do not understand RecA binding or its release from DNA following strand exchange, nor why it uses so much ATP. The structure of RecA protein is still to be determined. Other enzymes, notably those for resolving single-strand cross-overs and for mismatch repair in heteroduplex DNA, are needed to fill important gaps in the enzymology of genetic recombination.

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ARTICLES

Sedimentation under deep-sea storms

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Fast currents, high concentrations of suspended sediment and grooved mud beds are associated with erosion in frequent abyssal storms where a fast deep mean flow is augmented (or reversed) by intense intermittent currents. This occurs about 5 km below the Gulf Stream or its rings. The waning phase of a storm results in development of bedforms and rapid deposition of a mud blanket. Several other regions of the world ocean display evidence of abyssal storm activity.

REPEATED sampling of an area at 4.8–4.9 km depth lying under the path of the Gulf Stream or its warm-core rings revealed extremely high current velocities (up to 0.73 m s^{-1} (ref. 1)), suspended sediment concentrations up to 12 g m^{-3} (ref. 2) and bedform development. All these varied in time. The area is the lower continental rise south of Halifax, Nova Scotia (Fig. 1), and the measurements were taken as part of the High Energy Benthic Boundary Layer Experiment (HEBBLE)[3–5]. The experiment aimed to develop and test a coupled bottom Ekman layer and sediment transport model for cohesive sediment over uniformly distributed bed roughness[5]. Regional investigations in HEBBLE have provided numerous hydrographic[6,7], photographic[8], sedimentological[9], current velocity[1,10–12], light transmission[13–15], and suspended sediment[2,16,17] data. These have been used to select a 4 × 2 km box on the lower continental rise at 40°27′ N 62°20′ W where bed roughness is uniform, bed slopes are low (<1:100) and currents are intermittently fast (Fig. 1). The Nova Scotian Rise was first investigated because earlier data on sediments, photographs and currents suggested active sediment transport in the area[18–20].

Most sediment is carried to the depth of the continental rise by debris flow and turbidity currents. Subsequently, bottom currents produce two well-defined deposit types: those dominated by reworked current-deposited sediment, exemplified by parts of the eastern margin of the United States[21,22], and those where current reworking is slight, such as the margin off northwest Africa[23–26]. The lower Scotian Rise lies downstream of the Laurentian Fan, a major sediment accumulation produced by downslope sediment transport[27,28]. This is a probable source of fine material moving over the HEBBLE site in a deep western boundary current (which is not the 'western boundary undercurrent' comprising Norwegian Sea overflow water; this occurs above 4,000 m on the rise[10,29], Fig. 1*a*).

Here we draw together diverse data bearing on the rapid erosion, transport and deposition of sediments on the lower Nova Scotian Rise and on the driving forces, a combination of a strong mean component (DC current) with strong fluctuating components (AC current). We then examine the implications for sediment dispersal and deposition in the deep sea.

Sedimentology and stratigraphy

The 2 × 4 km HEBBLE box (Fig. 1*b*) was sampled with more than 30 box cores in July 1982. Over this area the upper unit is a ~4-cm layer of soft brown mud with a silt/clay ratio of 1.70, foraminiferal sand content of 6% and water content (% wet weight) of ~50.0%. The thickness of this layer measured in cores and on X-radiographs varies between ~0.5 cm and 12.5 cm. Its vane shear strength is uniformly low at ~0.4 kPa, rising rapidly to ~4 kPa in the underlying layer of foram clayey silt[30], whose water content decreases from 45.8% at the top of the layer to 36.9% at a depth of 14 cm. This underlying unit is about 30 cm thick, is extensively burrowed (live worms have been found down to the base of the unit) and is probably all Holocene in age as it contains *Globorotalia menardii*. The foram mud contains 1–30% sand, chiefly of planktonic foraminifera. Two longer cores have a gravelly mud of presumed late Pleistocene age beneath the Holocene.

Internally, the surface mud unit shown in Fig. 2 is variably burrowed and laminated. Some burrows are large and terminate in mounds, others are a filamentous network. The uniform physical properties of this surface layer suggest rapid deposition. This is corroborated by ^{234}Th ($t_{1/2}$ = 24 days) distributions showing penetration of the radionuclide to depths as great as 6 cm (ref. 31). A balance must be struck between introduction of this material by advection (sediment deposition) and (biological) mixing, parameterized as a diffusivity[32,33]. The best estimate

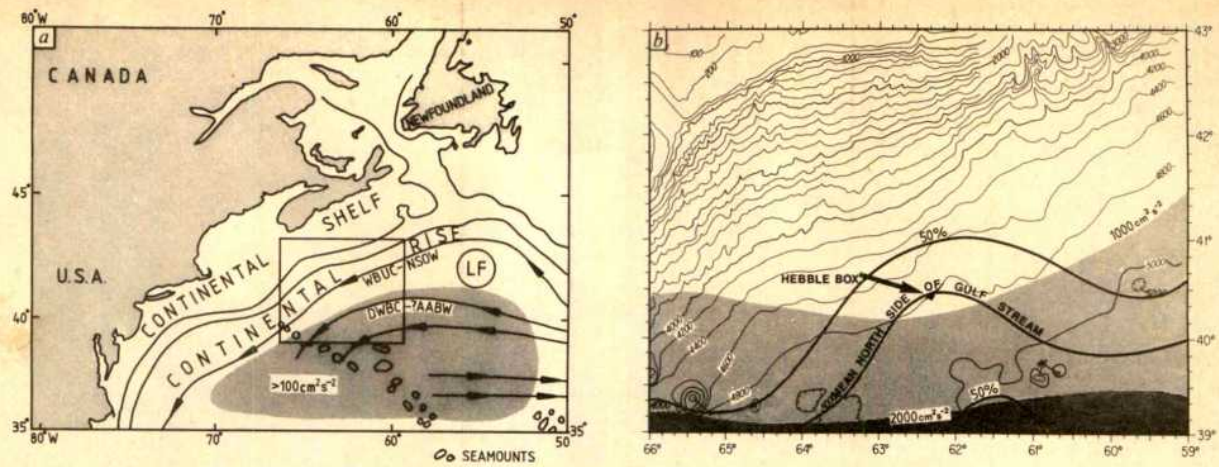


Fig. 1 Location of the HEBBLE box on the Nova Scotian continental rise. *a*, Physiography of the region with the paths of the Western Boundary Undercurrent (WBUC) transporting Norwegian Sea Overflow Water (NSOW) and a Deeper Western Boundary Current (DWBC) with cold ($\theta < 1.82$ °C) water of possible Antarctic Bottom Water (AABW) origin[6,29], and, stippled, the region of high abyssal kinetic energy ($>100\ cm^2\ s^{-2}$)[38,39]. *b*, Contoured enlargement of the slope–rise province with location of the HEBBLE box, the mean position of the northern side of the Gulf Stream[73] and its ±50% limits, and, shaded, the region of high surface kinetic energy ($>1{,}000\ cm^2\ s^{-2}$)[74]. Bathymetry of *b* courtesy of Dr A. N. Shor. LF, Laurentian Fan.

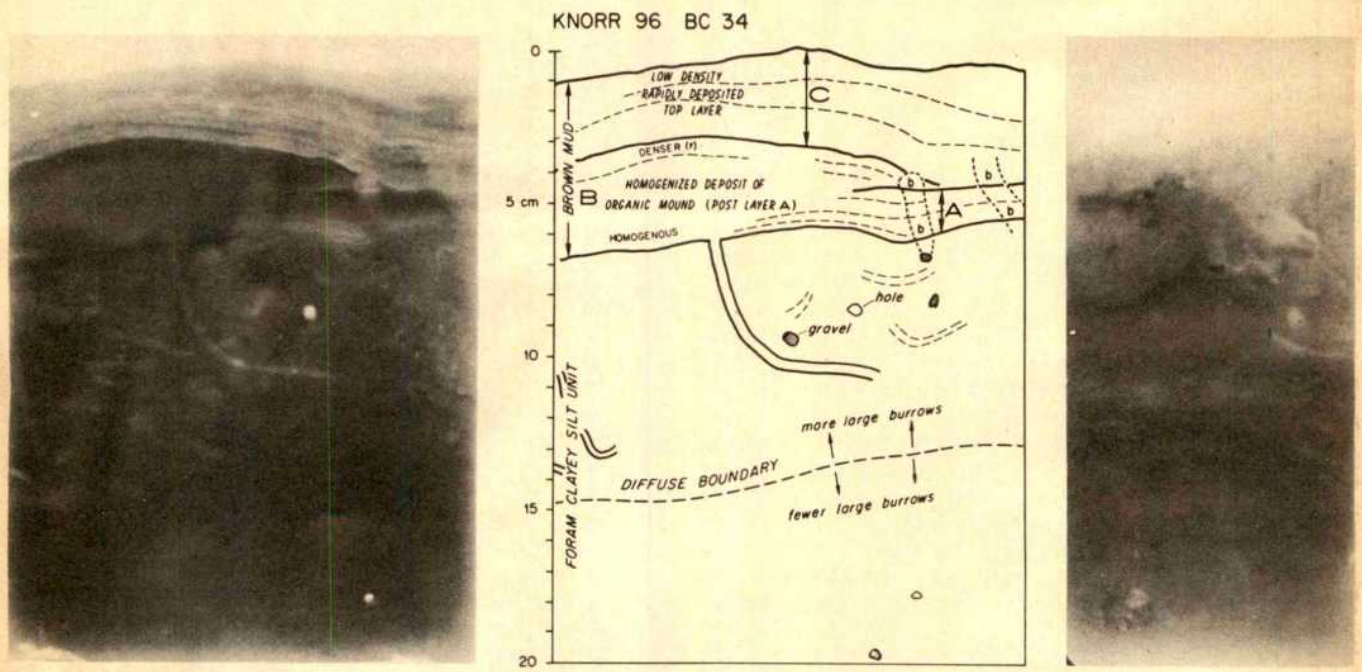


Fig. 2 Left: X-radiograph of the uppermost sediments in *Knorr* Cruise 96 (July 1982) box core 34. Centre: interpretation of the internal structure showing two depositional events (A and C) separated by an episode of mound construction (B). Right: an X-radiograph from box core 2 showing burrow-mixed upper part of the sediment column containing fine gravel (dark grains).

from one site is a deposition rate of 1.42 cm per month (17 cm yr^{-1}) and mixing parameter of 85 $cm^2\ yr^{-1}$ (ref. 30), values confirmed and extended by analysis of more samples by our colleagues D. DeMaster and C. Nittrouer[31]. These extremely high rates require very high suspended sediment concentrations (~100 g m^{-3}) occurring intermittently over a few months[30]. Deposition is often followed by rapid biological mixing of the sediment, a feature well shown by some X-radiographs (Fig. 2).

Detailed size analysis of 20 samples from 1–5 mm depths taken in July 1982 and June 1983 reveal a marked change in the material present. At depth, below the surface brown mud, on both occasions the silt fraction (2–63 μm) contained two modes at ~4 μm and ~14 μm. However, the surface 4 cm in July 1982 showed only a single peak at ~14 μm (ratio of % at 14 μm to % at 4 μm = 2.99 ± 1.38) whereas in June 1983 the bed was quite different with a clearly bimodal size distribution and a peak–height ratio of 1.42 ± 0.24 (±1 s.d.). The unimodal surface layer deposited before July 1982 must have been laid down in conditions of higher shear stress, suppressing deposition of the finer mode. Taking the preliminary *in situ* settling velocities (from trials of the optical tube of R. Zaneveld) for the fine mode to be 5.5×10^{-4} cm s^{-1}, the boundary shear stress under which the 1982 bed was deposited, preventing deposition of 4 μm silt, was >0.005 Pa (shear velocity >0.22 cm s^{-1}), while the 1983 bed was laid down under a lower stress[34]. Many X-radiographs reveal a laminated upper 5 cm of the bed, thus the bimodal deposit of 1983 could not have been formed by biological mixing-in of fine sediment deposited on top of the 1982 layer. We conclude that the bed of July 1982 had been completely eroded and that a new deposit of 'bimodal mud' up to 4 cm thick had formed since then. Again, our inference is

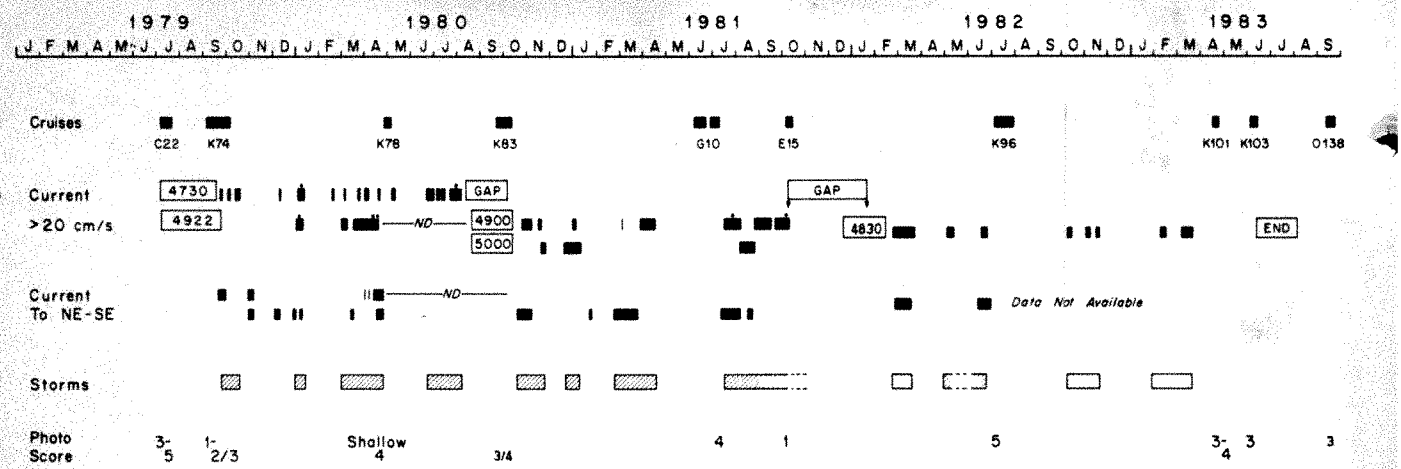


Fig. 3 A time-series of: *a*, cruises to the Nova Scotian rise in HEBBLE by Research Vessels C = *Conrad*, K = *Knorr*, G = *Gyre*, E = *Endeavor*, O = *Oceanus*; *b*, times when the currents were $>0.20\ m\ s^{-1}$ and, with a dot above the block, $>0.40\ ms^{-1}$ (courtesy of Dr M. Wimbush and Dr G. Weatherley; data for October 1980 to June 1982 in Weatherley and Kelley[6] and Koenig *et al.*[8]); *c*, times when currents flow to the east indicating eddy reversal of the mean flow, from the data sources for speed; *d*, times when the combination of speed and direction can be called a storm—this includes some lulls in the storm(s); *e*, scores (based on Table 1 criteria) of the suites of photographs from the vicinity of the HEBBLE box for cruise G10 onwards, for the deepest stations of K78, and for a 4,950 m station for K83. The range of C22 and K74 scores is discussed in the text.

one of extremely rapid erosion and deposition in this area, at rates which would not be thought low in an estuarine environment.

Photographs and currents

We have had at least one cruise per year to the Nova Scotian Rise since 1979, returning photographs from between 4,750 and 4,950 m (Fig. 3). Gross bedform zonation of the rise shows evidence of more tranquil conditions where the bed is shallower than 4,500 m and of faster currents below 4,750 m with very common longitudinal ripples below 4,850 m[8,35]. The zone between bottom depths of 4,750 and 4,950 and from 61 to 64°W was photographed in July and September 1979. These and the subsequent photographs can be assigned to a position in a rough scale of relative importance of erosion or deposition on the basis of the many features observed (Table 1) (compare with ref. 36, p. 357). It is not possible to calibrate this scale properly, though grooving (a score of 1) observed in October 1981 was associated with currents which exceeded $0.4\ m\ s^{-1}$ in the preceding 2 weeks (Fig. 3), and the tranquil end (a score of 5) has currents less than $\sim 0.05\ m\ s^{-1}$. It is unlikely that velocities for scores 2 to 4 can be obtained by linear interpolation.

The large-scale spatial variability displayed by the 1979 photographs also contains a strong temporal signal. In July the area was all at the depositional end of the scale, from 3 to 5 on the criteria of Table 1, with the scores of 4 and 5 occurring at shallower depths and 3 at depths over 4,900 m. In September the photographs were taken during a period of strong currents. Two of the stations between 61 and 61°30′ W at 4,840 and 4,920 m showed pronounced grooving, exhumed tubes and other indicators of erosion. Other stations rating scores of 1 to 2 occur along the south (deeper) side of the photographed region. The lowest scores at this time are 2 to 3 at 4,830 m depth. The most intense effects were seen in deeper water with intense scouring at two stations in the east of the region. A circular area incorporating the two scoured stations would be $\sim 10^3\ km^2$ but (the affected region might be more extensive along isobaths).

From 1981 onwards all photographs were precisely navigated by bottom transponder in the HEBBLE box. They display enormous temporal variability (Figs 3, 4), ranging from the most severe erosion to tranquil deposition with organic reworking, ratings of 1 to 5 on the criteria of Table 1. The end members are represented by the areas photographed in October 1981 on *Endeavour* 75 (score 1) and July 1982 on *Knorr* 96 (score 5) (Fig. 4*a*, *c*). Over an area the size of the HEBBLE box the photographed bed condition is spatially very uniform at a given time. During *Knorr* 96 the 16 sub-areas had over 20 photographs taken in each, and none rated a score less than 4. Current velocities measured about 35 km south of the EN 75 photographs during the previous 10 days (1–8 October) were all $>0.30\ m\ s^{-1}$ on daily average, and for 2 days they were $>0.40\ m\ s^{-1}$ (ref. 6). During *Knorr* 96 the photographs show surfaces with mounds, scattered faecal pellets, organic tracks and trails, various animal holes in the mud and occasional fine lineations. For the week before the start of the photographic survey, currents at 59 m above bottom (mab) in the HEBBLE box were $<0.10\ m\ s^{-1}$ and for half the time they were $<0.06\ m\ s^{-1}$.

At intermediate speeds, 0.05 to $0.15\ m\ s^{-1}$, deposition of silt occurred with development of barchan-like ripples in ~15 μm silt containing ~25% clay and <10% sand (Fig. 4*b*). The steeper faces on these bedforms suggest a measure of non-cohesive behaviour on the part of these clayey silts. The barchan ripples grow on the downstream side of biogenic mounds. It is not clear what dynamical condition controls the difference between formation of either single tails (mound-and-tail structure) or 'swallow-tailed' barchan ripples downstream of mounds. These

Table 1 Photographic features indicative of erosion/deposition

Strong erosion				Tranquil deposition
1	2	3	4	5
Scraped grooves				
Exhumed tubes				
Winnowed sand sheet				
Crag and tail				
	Barchan ripples			
	Cornices/slip faces			
	Lineations			
		Smoothed surface		
		Mounds and tails		
		5-Holes and fairy rings		
			Animal tracks	
			Ampharetid pits	
			Symmetric mounds	
			Clear water	
				Organic surface debris

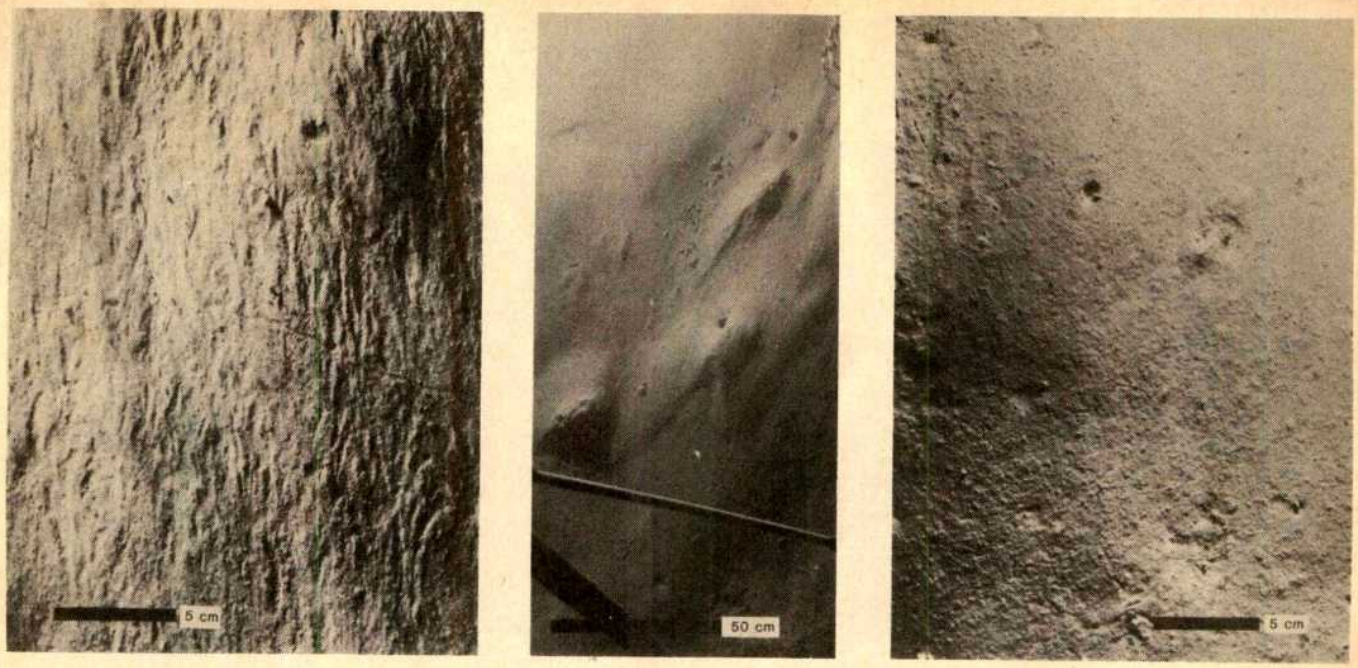


Fig. 4 Representative photographs for the conditions of: left, major erosion yielding a grooved bed (EN75); centre, deposition under a moderate current yielding barchan ripples in clayey silt (KN103); and right, tranquil conditions with a biologically textured bed and little evidence of current activity (KN96). All from the area of the HEBBLE box.

conditions are exemplified by the photographs of April and June 1983.

On the basis of the stronger bottom currents and direction reversals shown on Fig. 3 we have picked off certain times that we call 'storms'. We define as abyssal storms those periods of several days to a few weeks with intermittently high current speeds (over 0.20 m s^{-1} with peaks over 0.30 m s^{-1}) and often with reversals of current direction (see current meter records in Weatherly and Kelley[10]). Associated with this perturbation of the velocity field is high turbidity caused by resuspension of bottom sediments which some authors have termed storms[15,37]. We prefer to view the increased turbidity as a common consequence of storminess. There are about three of these storms per year occupying about 35% of the time. Less than a third of them contain speeds over 0.40 cm s^{-1} at the HEBBLE site, though they may contain such speeds somewhere in the vicinity. Nevertheless, an area can expect, on average, to suffer one of the intense scouring events like that photographed in October 1981, once per year, and be subject to a scale 2 or 3 event twice per year. The material put into suspension is deposited (or dispersed) in the remaining 65% of the time. The deposition is probably as episodic as the erosion, since concentration controls deposition rate both directly and through its control of settling velocity, thereby ensuring that initial deposition rates after storms will be fast.

The rates of deposition suggest that the upper layer 'C' of the sediment column shown in Fig. 2, 2.5–4 cm in thickness, was probably deposited between the minor storm of March 1982 and the sampling date of July 1982. The first layer 'A' probably post-dates the intense October 1981 storm, putting the mound formation in the period December to February 1982. These rates of up to a centimetre per month deposition are consistent with the ^{234}Th dating mentioned above[30,31]. The two-event signature of box core 34 (Fig. 2) is shown in at least 5 of the other 15 sub-areas of the HEBBLE box. The stratigraphy of the uppermost sediment column here is extremely ephemeral and its chance of being preserved very small. The overall Holocene deposition rate on this part of the continental rise is ~5.5 cm kyr^{-1} (giving an annual deposition/preservation ratio of 3,100), and the deposits preserved are almost totally disturbed by burrowing animals.

Driving mechanisms

The area we describe has three abyssal features possessed by several other points in the world ocean: (1) strong mean flow of 0.10–0.15 m s^{-1} (refs 10, 29); (2) high variability of flow speed and direction resulting in a high eddy kinetic energy (variance of speed)[38–40]; and (3) a large, fresh supply of mud in the form of turbidity current deposits located upstream (the Laurentian Fan). Several other sites with large supplies of mud and strong steady currents have been examined elsewhere, for example, Feni, Hatton and Gardar drifts in the northeastern Atlantic[41,42], and Blake and Greater Antilles Outer Ridge in the southwestern North Atlantic[43,44]. In none of these locations have the extraordinarily high turbidities (>1 g m^{-3}) and flow speeds attained on the Scotian Rise been matched.

The high turbidities attained must be related to high rates of erosion, as Armi[45] shows that they are not due to turbidity currents, an earlier explanation[46]. An important determinant of this is the sediment water content[47,48]. Rapidly deposited sediments have high water contents, and in the HEBBLE area the water content of the surface 2 cm ranges from >100% dry weight (>50% wet weight) to 170% (61% wet weight) (J. Y. Yingst and A. J. Silva, personal communication). This high potential erodability is a consequence of the fluctuating velocity regime, which allows rapid deposition of material under the low velocities following a storm. By contrast an area which suffers a more sustained stress may become 'stress-hardened' and more difficult to erode, requiring a large fluctuation in speed to move it[49,50]. Of course, the fact that the maximum currents here are up to 0.73 m s^{-1}, almost twice as high as those flowing over other deep-sea sedimentary bodies, also helps cause rapid erosion.

The origin of the high abyssal eddy kinetic energy in this region appears to be related to the high surface eddy kinetic energy of the Gulf Stream overhead[38–40,51,52]. However, Weatherly[39] shows that proximity of the Gulf Stream or its warm-core rings to the deep current meter locations is associated with a high frequency of current reversals (easterly flows) and with mean speeds of 0.15 to 0.20 m s^{-1} to both west and east. Rhines'[53] numerical models suggest a uniform decrease of the ratio of surface to bottom velocity with increasing E/δ where

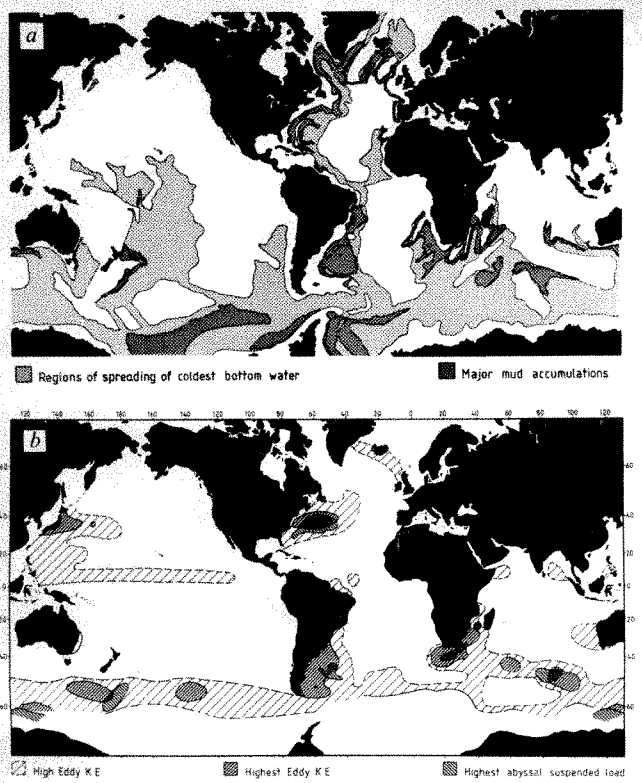


Fig. 5 *a*, Regions of spreading of cold bottom water[67] (light stipple) superimposed on distribution of large current-controlled accumulations of mud[68] (heavy stipple). *b*, Areas of high surface eddy kinetic energy or variability of surface elevation, based on Wyrtki *et al.*[71] and Cheney *et al.*[69], with, superimposed, the region of highest suspended particulate load ($>20\,g\,m^{-2}$) in the North and South Atlantic from Biscaye and Eittreim[64], the maximum turbidity in the Indian Ocean from Kolla *et al.*[65] and the turbidity high south of New Zealand from Dr P. E. Biscaye.

E is the Rossby number and δ is the relative roughness, a feature demonstrated by Dickson's results[40]. At high energy levels the eddies should be more barotropic[40] and the flow should depend only weakly on depth as shown in the Gulf Stream area by Schmitz[54]. Looking at the western Atlantic as a whole, Schmitz[38] identifies regions of deep strong mean flow as ones of enhanced eddy kinetic energy though the values are only up to half those found under the Gulf Stream.

Implications

(1) The deep-sea stratigraphical record under sites of high eddy kinetic energy is much more fragmentary than generally supposed. The situation more closely resembles storm-dominated shelf sedimentation than the continuous accumulation presumed for many parts of the deep ocean. In the Scotian Rise area the mean deposit thickness of 10^3 yr net accumulation can be removed in a few weeks and redeposited in a few months. The high nutrient flux resulting from both horizontal advection and vertical flux from the Gulf Stream's surface productivity allows intensive activity by burrowing organisms[9], yielding phenomenally high values of biological diffusivity ($3–85\,cm^2\,yr^{-1}$)[30,31] compared with previous deep-sea values (all $<0.5\,cm^2\,yr^{-1}$). Thus the signal of intermittent erosion and deposition is largely smeared out or erased once the sediments have passed below the zone of episodic erosion.

(2) This region is clearly important for generation of nepheloid layers and for their thickening by detachment and injection of turbid bottom-mixed layers to points higher up in the water column[13,16]. The high horizontal eddy kinetic energy may also be accompanied by enhanced vertical eddy kinetic energy and thus higher vertical turbulent diffusivity. It has been suggested that the cross-isopycnal diffusivity of $1–3\,cm^2\,s^{-1}$ identified by Hogg *et al.*[55] and Whitehead and Worthington[56] also results in the thickening of the nepheloid layer above the bottom-mixed layer through upwards diffusion of small particles[16,57]. A major feature of the GEOSECS suspended sediment profile down the western Atlantic is relatively high concentration extending up to a depth of 2,000 m (>2,000 m.a.b.) between 35° N and 45° N[58]. This may contain material resuspended from the bed under high eddy kinetic energy of the Gulf Stream and warm-core rings on the continental rise or cold-core rings over northern Bermuda Rise, which has been diffused upwards, as well as sediment that has spread laterally along isopycnals. Below the surface layer the small particles (mainly $<5\,\mu m$) providing most of the light-scattering signal to deep-sea nephelometers have a long residence time in suspension, probably more than a year[16,59]. Significant amounts of the optically detected material in nepheloid layers may thus have an origin in resuspension far from the detection point. This point is supported by the chemical data of Bishop and Biscaye[60], who find similarity between suspended and bed material in areas of strong currents where resuspension is likely, but some dissimilarity in quiescent regions with weak nepheloid layers. There the bottom sediment results mainly from rapid vertical flux from the surface and the nepheloid layer contributes only a slight rate of deposition. The stormy areas of the sea bed are seen as major sources of suspended material which is raised up and then advected across ocean basins. This situation is roughly analogous to that of soil aerosols, a major portion of which originate from a few areas with erodible soils and intermittently strong winds[61,62].

(3) The points of major resuspension under abyssal storms in the world ocean are likely to be those where surface eddy kinetic energy is high and coupled to a strong near-bottom mean flow and sufficient mud. An extremely dense nepheloid layer would indicate the possibility of storm activity and large sediment drifts and mud waves indicate significant long-term deposition. Several authors using the Lamont nephelometer have demonstrated a clear relationship between the regions of high mean velocity in deep boundary currents and high suspended sediment content[63–66]. Regions of spreading of coldest bottom water[67] and areas of depositional sedimentary bedforms[68] correspond closely (Fig. 5*a*). The distribution of eddy kinetic energy inferred from satellite[69,70] and ship drift observation[71] shows several areas of elevated surface eddy kinetic energy and a few particularly high spots where we presume high abyssal eddy kinetic energy. These are the North American basin under the Gulf Stream system, the Argentine basin under the confluence of the Falkland and Brazil currents, under the Kuroshio off Japan, in areas off South Africa in the Natal Valley and Mozambique Basin beneath the Agulhas current, in the Tasman Sea under the East Australian current, and over large areas beneath the Antarctic Circumpolar Current—south of the Crozet Plateau, east of the Kerguelen Plateau, in the Weddell Sea and on the southwestern side of the New Zealand Plateau (Fig. 5*b*). Many of the areas of potential bottom storminess are also the regions of maximum turbidity in the ocean basins (Fig. 5*b*). In the Atlantic the two maxima are under the Gulf Stream and in the Argentine Basin[63,64]. In the Indian Ocean maxima coincide with the Agulhas region and the area east of Kerguelen[65]. South of New Zealand along the southern end of Chatham Rise, P. Biscaye (personal communication) has located the maximum turbidity in the Pacific. The notable exception to this list is the area under the Kuroshio where no great source of suspended material has been detected[72]. The large amount of sediment eroded from Japan goes into the Japan trench and there is no strong near-bottom thermohaline flow in the region. The Pacific equatorial high surface kinetic energy does have a substantial accumulation of biogenic mud beneath it, but neither there nor in the Indian Ocean are there strong thermohaline currents along the bottom. Although only two areas under the Antarctic circumpolar current have demonstrated extremes of turbidity there is a general paucity of data from the region. It may be among the most stormy (at the bottom as well as the top) in the ocean and act as a major source of suspended material to

the abyssal thermohaline flows emanating from there and penetrating the western sides of the ocean basins.

These results drive us towards a fundamental revision of the notion that "... the warm water sphere protects and isolates the abyss from the more familiar forces of weather, leaving as the only significant dynamic factors in the nearly closed abyssal system the thermohaline currents ...' (ref. 36, p. 371).

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Fractal growth of copper electrodeposits

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Black dendritic growths of copper were electrodeposited in steady-state diffusion-limited conditions onto an initially point-like cathode. Their spatial extent R *was inferred from the increased current and their mass* M *from the total charge transferred. Fractal behaviour* $M \propto R^D$ *was observed with* $D = 2.43 \pm 0.03$*, in good agreement with computer simulations of diffusion-limited aggregation.*

IF the accretion of material onto a surface is limited by diffusion onto the surface, deposition occurs preferentially on any protuberances (just as electric field lines concentrate on the convexities of a lightning conductor). In this way any initial roughness grows exponentially—the Mullins–Sekerka (MS) instability—and the deposit rapidly acquires a dendritic quality. Surface properties limit how finely ramified the deposit grows. This instability has been long recognized by materials scientists, with examples ranging from alloy solidification patterns to dendritic crystal growth (see ref. 1 for a review).

Recently, Witten and Sander[2] introduced a simple computer model in which particles diffuse onto a cluster by random walk, one at a time. This model differs from experimental systems principally by the absence of any surface tension or crystalline factors and by the very slow rate of reaction; and in this 'diffusion-limited aggregation' (DLA) model the clusters grow immediately dendritic and disorderly. They were shown to be fractal[3] in the sense that inside a large cluster the number of particles within a radius R of any given particle was on average proportional to R^D. The index D is the Hausdorff dimensionality[4] of the deposit and was found by computer simulation in three dimensions by Meakin[5] to be $D = 2.495 \pm 0.06$. Note that this model does not seem to be applicable to experiments (ref. 6 and D. Weitz, in preparation) where clustering of clusters was allowed and $D = 1.7$ obtained.

Even in the experimental systems where the MS instability is

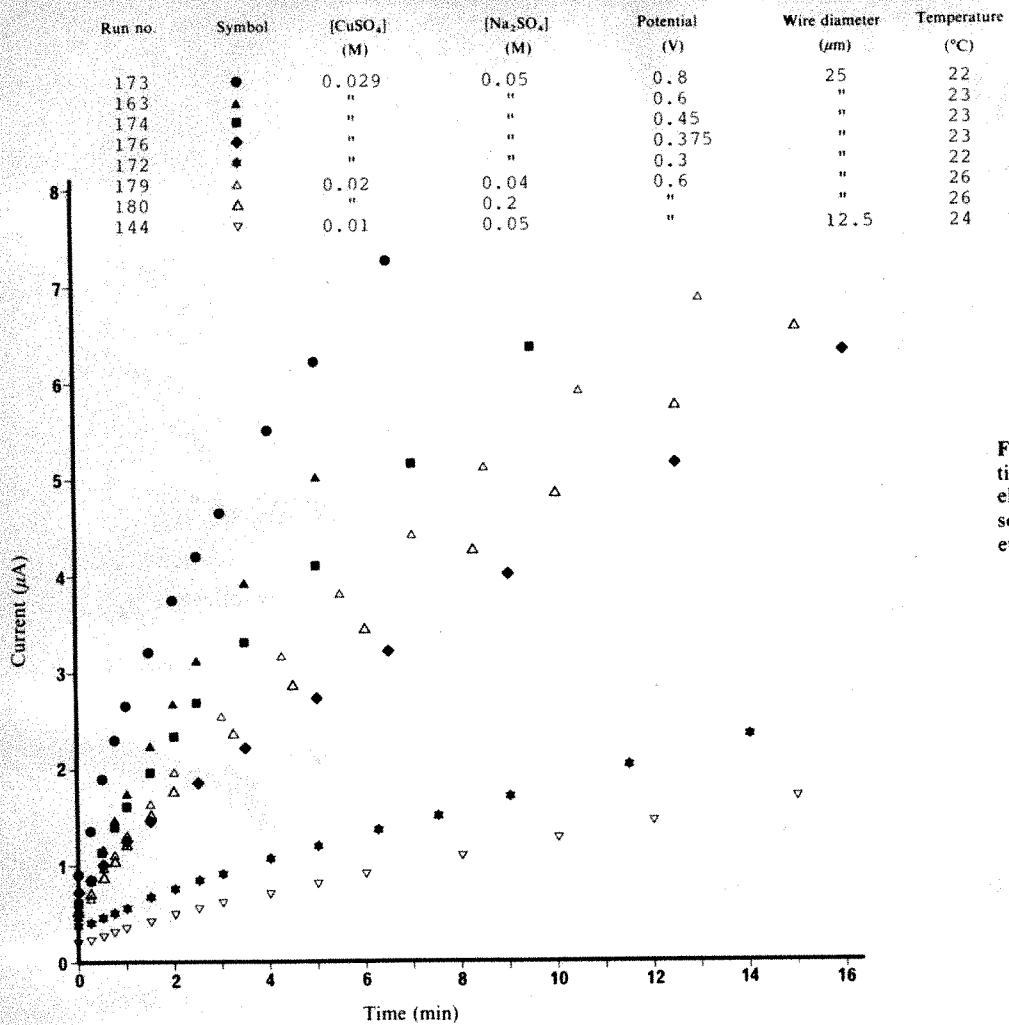


Fig. 1 Increase of current with time, for various potentials and electrolyte concentrations. All the solutions contained 1.4 wt% polyethylene oxide to impede convection.

limited by surface properties, Witten and Sander[2] suggested that weak noise should ultimately lead to disorder and similar fractal behaviour. Thus, at short length scales there may be local order due to surface tension or crystallographic factors, but at longer length scales they suggested random fluctuations should dominate: in particular, the Hausdorff dimensionality D found by computer simulation should also characterize experimental systems at long length scales.

We now report an experimental measurement of the Hausdorff dimensionality D of copper deposited electrolytically from aqueous solution in diffusion-limited conditions, onto an initially point-like cathode. The deposits were grown larger than the initial cathode but smaller than any diffusion length in the system. Because the diffusion-limited current onto such a spherical absorber is proportional to its radius (and[7] not its surface area), the instantaneous current gave an effective overall radius R_s for the deposit. With the mass M of the deposit determined from the total charge transferred, the relationship between M and R_s could then be inferred, and the fractal law $M \propto R_s^D$ verified.

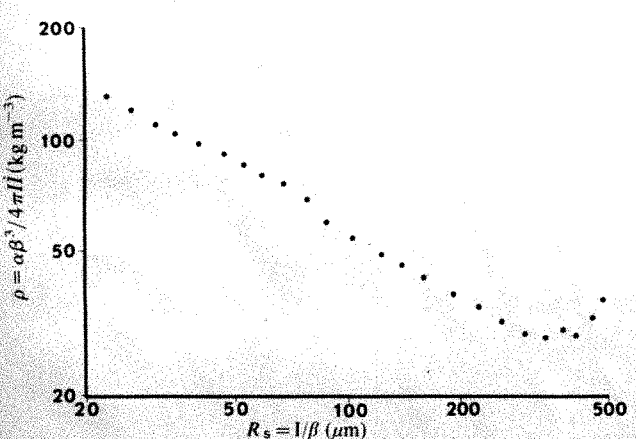


Fig. 2 Logarithmic plot of inferred density as a function of radius, for run 144.

Experiment

Solutions of copper sulphate and sodium sulphate were made up using deionized, distilled water. Polyethylene oxide $(C_2H_4O)_n$ with molecular weight 6×10^6 was added at 1.4 wt%, increasing the viscosity by a factor of 1,000 to ~1 P. The solutions were stored away from light and vigorous stirring was avoided, to prevent damage to the polymer.

In each experiment a sample of the solution was placed inside a hole of diameter 7 mm drilled in a copper block, which acted as the anode. Insulated wire of diameter 12.5 or 25 μm, the end freshly cut with a razor blade, acted as the cathode. The copper deposit would grow in a dendritic cluster around the cut end of the wire which was suspended vertically into the solution. To prevent mechanical movement during the experiment the free length of wire was kept to <7 mm, a vibration-free mounting covered with a bell-jar was used, and at least 5 min were allowed for any movement to die down before the voltage was applied.

The experiments were performed at constant voltage set in the range 0.3–0.8 V, and the current through the cell was measured as a function of time. Initially the interval between readings was 10 s, increasing to several minutes as the current rose more slowly. Typically the experiments were stopped after half an hour.

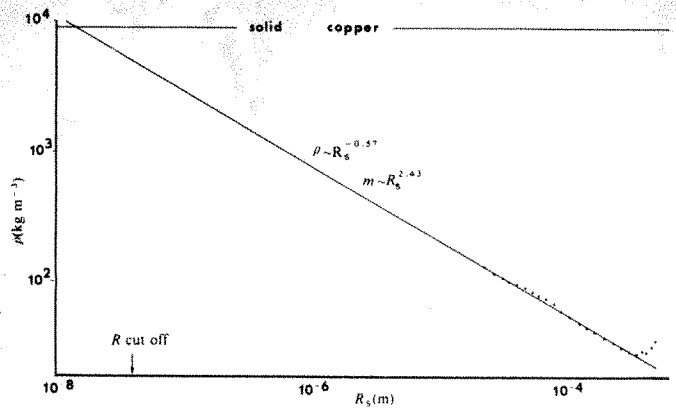


Fig. 3 The data of Fig. 2 plotted on a scale showing the density of solid copper and the radius R_{cutoff} of crystallites observed in electron micrographs. Note the extrapolation (solid line) corresponds to a Hausdorff dimensionality of 2.43.

Figure 1 shows direct plots of current I against time t for various voltages and electrolyte concentrations. It can be seen that the current grows much faster for higher voltages and higher concentrations of copper sulphate, but is relatively insensitive to the sodium sulphate concentration.

It was noted that changing the instantaneous voltage did not change the instantaneous current significantly in our experiments. Any change would have indicated that the reaction was not limited by diffusion of Cu^{2+} ions but was dominated by some other mechanism. The set voltage thus influenced the current–time curves through the morphology of the deposit and not directly through any ohmic or activation limit on the conduction.

Figure 2 shows, with interpretations discussed below, the rate of change of current dI/dt plotted as $-\log I(dI/dt)$ against $\log I$. Plotted in this way the experiment was reproducible to better than 10% except at high currents. Problems at high current and hence large radii included mechanical motion (for example, the wire bending under the weight of the deposit), stirring of the solution, and contact resistances (later improved by applying silver dag).

Results and interpretation

We define an effective radius for the deposits in terms of the diffusion-limited current. Modelling the cathode growth by a perfect spherical absorber of radius R_s, the current[7] is

$$I = \beta R_s \tag{1}$$

the coefficient β being given by

$$\beta = 4\pi KCq \tag{2}$$

where K is the diffusion coefficient, q the charge, and C the number density far from the absorber of the copper ions. This 'Smoluchovski radius' R_s characterizes where the deposition is taking place, and thus how far out the deposit extends in space. Clearly R_s will be of the same order of magnitude as the radius of gyration, R_g, measured in computer simulations[2,5]. Indeed, provided the growths are self-similar fractals as the simulations suggest, within the diffusion-limited regime the ratio R_s/R_g will be a constant independent of all parameters.

The number N of copper atoms deposited can be inferred from the charge transferred Q,

$$N = Q/q = \alpha \int I\,dt \tag{3}$$

Using equations (1)–(3), the relationship between the radius R_s and the number of atoms N can in principle be observed directly throughout the growth of the deposit. In practice the cathode was initially of finite size so that there would be an offset error in the N obtained by integration. It is to avoid this that we have instead plotted dI/dt in Fig. 2.

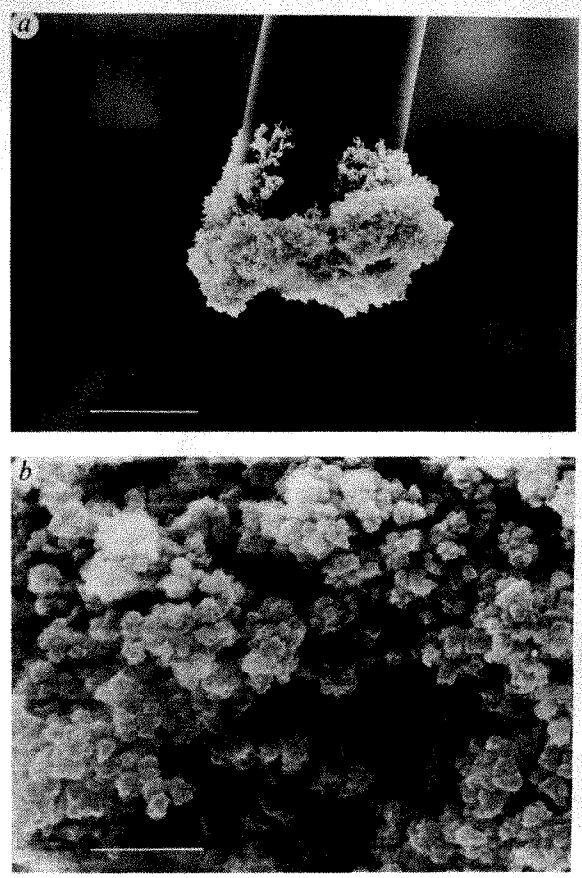


Fig. 4 Electron micrograph of a deposit grown for only 1 min in the absence of polymer. 0.01 M $CuSO_4$, 0.05 M Na_2SO_4, 0.6 V. Scale bars: *a*, 20 μm; *b*, 1 μm.

The quantity

$$\rho = \frac{\beta^3 \alpha}{4\pi I \dfrac{dI}{dt}} \tag{4}$$

may be interpreted as an effective density of deposition since, using the definition (1) above,

$$dN = 4\pi R_s^2\, dR_s \rho \tag{5}$$

Figures 2 and 3 show the density–radius plot inferred from the data shown in Fig. 1. Note the logarithmic straight line $\rho \propto R^{-0.57}$ which implies fractal behaviour, $N \propto R^D$ with $D = 2.43$. Writing this result in the form

$$\rho = \rho_{Cu}(R/a)^{D-3} \tag{6}$$

where ρ_{Cu} is the density of solid copper, the length a characterizes the onset of fractal behaviour. Extrapolating the data in Fig. 3 back to the solid copper density gives an intercept of

$$a = 14\ \text{nm} \tag{7}$$

which compares with the characteristic radius of the dendritic features seen in the electron micrographs (Fig. 4)

$$R_{cutoff} = 40\ \text{nm} \tag{8}$$

The specimen in the micrographs was produced in similar conditions, except that it was grown for only 1 min and with no polymer present so as to minimize damage on removal from the solution.

The experiment was repeated at various electrolyte concentrations as listed in Table 1 together with the Hausdorff dimensionalities D obtained. The values of D show no evidence of systematic variation and taken together give

$$D_{\text{experiment}} = 2.43 \pm 0.03 \quad (9)$$

which compares with the result from DLA computer simulations[5]

$$D_{\text{DLA simulation}} = 2.495 \pm 0.06 \quad (10)$$

For each run the intercept a was extrapolated using the value $D = 2.43$, and Fig. 5 shows how the reciprocal radius $1/a$ varied with the voltage of the experiment. We have no explanation for the apparent law,

$$1/a \propto V - (0.23\ \text{V}) \quad (11)$$

Note that the intercept a in Fig. 5 does not depend on the concentration of copper sulphate.

Electrochemical considerations

Our interpretation of the experiment presupposes that the net reaction at each electrode was

$$Cu^{2+}(aq) + 2e^- \rightleftharpoons Cu(s) \quad (12)$$

and that the reaction was limited by diffusion of Cu^{2+} ions in the vicinity of the cathode.

To evolve hydrogen gas at the cathode in the principal experimental conditions (0.01 M $CuSO_4$, 0.05 M Na_2SO_4, neutral pH) requires at least 0.685 V (ref. 8). Below this limit there are no anion oxidations available, and the only remaining chemical difficulty is the role of singly charged Cu^+ ions. In experimental conditions there is an equilibrium ratio in the presence of copper (see ref. 8),

$$[Cu^+]/[Cu^{2+}] = 7 \times 10^{-3}\ (25\ ^\circ\text{C}, 0.01\,\text{M}\ Cu^{2+}) \quad (13)$$

and such a concentration of Cu^+ is soluble. With the anode working at low current density (of the order of 1 μA cm^{-2}), we presume that this equilibrium was maintained and therefore that the current due to Cu^+ ions was negligible.

If stirring of the solution is prevented, the electric fields will be screened and deposition will be limited by diffusion in the neighbourhood of the cathode. The calculated Debye–Huckel screening length for 0.05 M sodium sulphate is $\lambda = 1$ nm. (There is in fact a small unscreened voltage of the order of

$$V = kT/e([Cu^{2+}]_{\text{bulk}} - [Cu^{2+}])/[Na_2SO_4] = 5\ \text{mV} \quad (14)$$

required to maintain neutral charge density as the Cu^{2+} ions adopt a density profile governed by diffusion kinetics.)

Data from the Smithsonian Tables[9] indicate an ionic diffusion constant for aqueous Cu^{2+} at 24 °C of $K = 6.17 \times 10^{-10}$ m^2 s^{-1}, with a temperature coefficient of 0.0229 K^{-1}. This implies a value for our parameter β (equation (1)) of

$$\beta = 0.0121 \quad \text{A m}^{-1}\ (\pm 5\%) \quad (15)$$

We assume that the polymer had little effect so that the main error in the value for β was the use of dilute solution approximations.

Diffusion lengths

The assumption of Smoluchovski kinetics (equations (1), (2)) requires that the cathode radius R is less than any intrinsic diffusion lengths limiting the scope of a laplace equation for $[Cu^{2+}]$.

The rate at which the diffusant concentration can relax determines a diffusion length $\xi_t = \sqrt{Kt}$. From equations (1)–(4) the dimensionless ratio $\gamma_t = R/\xi_t$ is of the order of

$$\gamma_t = \sqrt{C/\rho} \quad (16)$$

reaching a maximum value of 0.1 for the largest deposits grown (see Fig. 3) with a concentration of copper suphate $C = 0.01$ M.

The fluid close to the cathode is depleted of copper ions and is thus less dense by about 2% than the surrounding fluid in

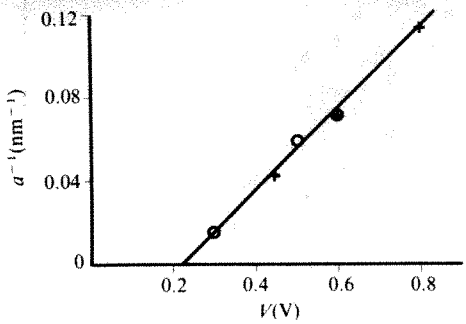


Fig. 5 Variation with voltage of intercept radius a. 0.05 M Na_2SO_4, 1.4 wt% polyethylene oxide. Concentration of copper sulphate (redetermined by solution conductivity): +, 0.029 M; ○, 0.01 M.

Table 1 Fractal dimension of copper electrodeposited from solution at 0.6 V

Run no.	$[CuSO_4]$ (M)	$[Na_2SO_4]$ (M)	Measured dimension
144	0.01	0.05	2.43
146	0.01	0.05	2.425
150	0.01	0.05	2.465
153	0.01	0.05	2.485
177	0.02	0.04	2.38
178	0.02	0.2	2.44
179	0.02	0.04	2.425
180	0.02	0.2	2.41
Mean dimension			2.433 ± 0.03
Computer prediction[5]			2.495 ± 0.06

The solutions contained 1.4 wt% polyethylene oxide to increase viscosity.

experimental conditions. Comparing the resulting flux due to convection with that due to diffusion gives a dimensionless parameter

$$\gamma_c = R/\xi_c = mgCR^3/\eta K \quad (17)$$

where mg is the weight of a copper ion and η is the viscosity of the solution. This gives a room temperature value $\gamma_c = 0.02$ for twice the radius of the thinnest wire used ($R = 25$ μm) if no polymer is added. Because $\gamma_c \propto R^3$ this would leave less than one decade of radius before convection became important.

The addition of 1.4 wt% polyethylene oxide raised the overall viscosity by a factor of ~1,000 without significantly affecting the diffusion of small inorganic ions. The range of diffusion-limited behaviour is thus extended in radius by an order of magnitude and the damping of mechanical disturbances also improved.

Conclusions

The two principal conclusions are that the electrolytic deposit of copper in experimental, diffusion-limited conditions is fractal, and that the Hausdorff dimensionality agrees closely with the simple computer model of diffusion-limited aggregation[2,5]. This is the first experimental verification of the conjecture by Witten and Sander that such fractal behaviour should occur at long length scales irrespective of local details. The experimental deposits were highly ordered up to order 40 nm, but the Hausdorff dimensionality was measured over the range 25–400 μm. Extrapolating the experimental data and comparing with electron micrograph observations suggests that in fact the fractal behaviour extends over the full range 40–400,000 nm.

There are several possible sources of systematic error in the present experiment. Convection, agitation, contact resistance and resistance and rigidity of the deposit were all problems that we believe were brought under control. Other possible problems

include non-inertness of the polymer, and the presence of any surface-active impurities.

In future work there are four aspects of the experiment which could be improved or varied: the type of measurement made; the electrolyte solution; the geometry; and the physical regime.

That the surface area of the deposit is proportional to its mass could be verified by measuring the capacitance. If the screening length in the solution is short and resistances can be rendered small or corrected for, the capacitance should be proportional to surface area.

The complications associated with the two possible oxidation states of copper might be avoided by, for example, the use of silver electrolytes. Convection might be suppressed without resort to polymer by the use of aqueous Ni^{2+} in the presence of excess CsCl: with these species there is the possibility of arranging that there be no net density change of the solution near the cathode. Other viscosity enhancers might also be tried.

It might be possible to make measurements in a two-dimensional geometry by, for example, using filter paper soaked in the electrolyte. In three-dimensional experiments, the use of microelectrodes would allow measurements to be made down to smaller length scales, but there could be mechanical problems with such an arrangement. A novel and more stable geometry would be to grow from a 'pinprick' (perhaps made by a spark) in the insulation on a plate: it would be useful to perform the corresponding computer simulation for growth from a point seed into the half space above a plane.

In our experiments the deposits grew slowly compared with the diffusion of ions. It should be possible by using higher electrolyte concentrations to explore the region where the deposits grow more quickly and behaviour is controlled by the diffusion length[1,10]. An alternative regime leading to fractal deposition might be obtained by stirring the solution until the reaction becomes limited not by diffusion but by ohmic resistance. It would then be the equations of electrostatics which drive the MS instability at long length scales.

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Two protein-binding sites in chromatin implicated in the activation of heat-shock genes

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The resistance to exonuclease digestion of two regions of chromatin at the 5′ end of heat-shock genes in Drosophila *implies they have protein bound to them. The pattern of resistance before and after induction of gene expression suggests that heat-shock genes are activated by the sequential binding of at least two protein factors.*

THE continuous array of nucleosomes along the eukaryotic chromatin fibre is punctuated by local discontinuities which are hypersensitive to endonuclease attack. Discovered originally at the origin of replication in the simian virus 40 (SV40) minichromosome[1–4] and in cellular chromatin encompassing the 5′ end of several *Drosophila* heat-shock genes[5,6], nuclease hypersensitive sites spanning about 100–300 bp of DNA in chromatin have since been widely observed (see refs 7, 8 for recent reviews). Usually, but not always, the hypersensitive sites are located near the 5′ end of genes. Their appearance precedes the onset of transcription and is also tissue- or stage-specific in developmentally regulated genes. The extreme susceptibility to nuclease attack is generally considered to indicate a local opening in chromatin for regulatory proteins and enzymes to enter and bind to target DNA sequences.

I have developed an *in situ* exonuclease III (Exo III) protection technique which maps sequences within nuclease hypersensitive sites in chromatin onto which regulatory proteins might be bound. Short stretches of DNA amidst the 5′ hypersensitive sites of the genes for the heat-shock proteins of molecular weight 83,000 and 70,000 (hsp83, hsp70) in *Drosophila* are indeed found to be resistant to exonuclease digestion. The resistant regions cover the TATA box sequence and an upstream control element.

5′ hypersensitivity of the hsp70 gene

Hsp70 is the most abundant of the small group of proteins inducible in *Drosophila* and other organisms by heat shock or other environmental stress[9]. There are five copies of the uninterrupted 2.2 kilobase pair (kb) hsp70 coding sequence, located at two chromosomal loci, 87A and 87C. The hsp70-coding region and about 350 base pairs (bp) of 5′ flanking sequences are highly conserved among all five genes[9–11].

Fine-structure analysis of the DNase I hypersensitive site at the 5′ end of the hsp70 gene gives an initial hint for a resistant region buried within. Nuclear chromatin from noninduced *Drosophila* cells (Schneider line 2, SL2) is digested gently with DNase I. After purification, the DNA is cleaved with a restriction enzyme, *Bam*HI, electrophoresed on a long agarose gel, blotted to nitrocellulose, and hybridized to a ^{32}P-labelled, probe segment chosen to abut the common *Bam*HI site at position +1,262 in the hsp70 gene sequence; +1 is the transcriptional start (Fig. 1). This effectively labels subfragments extending from that restriction site to the DNase I break made in the initial digestion;

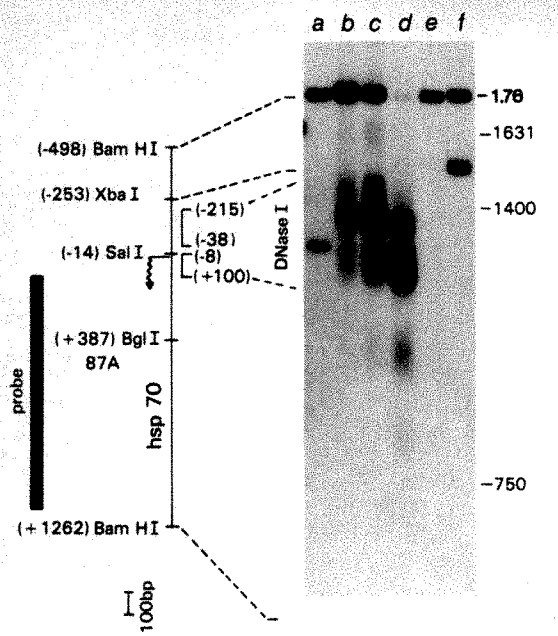


Fig. 1 DNase I hypersensitivity at the 5′ end of the noninduced hsp70 gene. A partial restriction map of one of the two hsp70 genes at chromosomal locus 87A is shown[11]. The start site (+1) and direction of transcription is indicated by the arrow.
Methods: Nuclei from *Drosophila* SL2 cells were isolated and digested with DNase I at 6, 8 and 4 U ml^{-1} (a separate reaction) respectively as described previously[5] (lanes *b–d*). Purified DNA (10 μg per lane) was restricted with *Bam*HI, electrophoresed on a 40 cm long, 1.4% agarose Tris-acetate EDTA (TAE) gel, blotted, hybridized to a ^{32}P-labelled, cloned probe fragment (solid bar) and autoradiographed as described previously[5]. For markers, SL2 DNA was restricted partially with *Sal*I, *Bam*HI and *Xba*I respectively, followed by complete *Bam*HI restriction and processing as above (lanes *a*, *e*, *f*).

thus the DNase I break points can be positioned by simple length measurement (the indirect end-labelling method)[5,12]. The probe used detects the *Bam*HI fragments and cleaved subfragments from all five hsp70 genes, although only the 1.76-kb *Bam*HI fragment corresponding to one gene is included in Fig. 1; the larger *Bam*HI fragments have been cropped away[5]. As noted before, the DNase I cleavages in chromatin span a broad region around the 5′ end of the hsp70 gene, although some suppression and enhancement is now clearly evident (lanes *b–d*). For precise mapping, genomic DNA restricted additionally at landmark sites are electrophoresed in adjacent lanes (lanes *a*, *f*), or are co-electrophoresed with the samples (data not shown). Thus the hsp70 sequences hypersensitive to DNase I in chromatin extend from positions +100 to −8 and from −38 to −215, with a peak at −93. The 30-bp region in between, from −8 to −38, is relatively resistant. These measurements are precise to 5–10 bp. The complex digestion pattern could be due to different contributions from each of the five hsp70 genes. However, it is qualitatively unchanged when the genes at either chromosomal locus are removed with specific restriction cleavage (data not shown); thus the average pattern is likely to represent each hsp70 gene. The site from −8 to −38 that is resistant to partial cleavage suggests bound nonhistone protein. For more conclusive evidence, a new test has been devised which can visualize the resistant fragments positively after limit nuclease digestion.

Mapping Exo III-resistant sites

One way to locate the borders of protein-bound sites on a piece of DNA is to nibble in from either end with an exonuclease, usually *Escherichia coli* exonuclease III[13], until further passage of the enzyme is blocked, and to measure the length of the resistant DNA strand in such a limit digest. This approach was used successfully to localize protein-binding sites in reconstituted complexes of DNA with purified *E. coli* RNA polymerase, λ integrase and SV40 T antigen, and to analyse the disposition of DNA in native and reconstituted nucleosomes[14–26]. Here I combine the use of Exo III with the indirect end-labelling method to detect the presence of specific barriers against Exo III digestion in chromatin.

As Fig. 2*A* shows, an initial cut is made within a hypersensitive site by a (partial) endonuclease reaction on isolated nuclei. This creates a free end from which Exo III can digest 3′ to 5′ along the DNA until a specific block is encountered in the limit. The DNA is purified and the single-stranded tails are trimmed with S_1 nuclease. After further cleavage with flanking restriction enzymes it is possible to determine the relative start and stop positions of Exo III by indirect end-labelling the subfragments produced from endo- and exonucleolytic cleavages.

For the noninduced hsp70 gene, the initial cuts can be made by each of four different endonucleases: DNase I, micrococcal nuclease, a Ca^{2+}–Mg^{2+} stimulated endogenous nuclease, and a restriction enzyme *Xho*I which cleaves at position −194, inside the distal boundary of the DNase I hypersensitive site (Fig. 2*B*). Exo III is added in increasing amounts simultaneously except for micrococcal nuclease, which is inactivated with EGTA without affecting Exo III added subsequently. The purified and S_1-trimmed DNA is restricted with *Bam*HI and *Xba*I, and the relative positions of the endo- and exonuclease cleavages are located by indirect end-labelling.

The complex pattern of 5′ hypersensitivity is again observed when only the initial cuts are made with no added Exo III (Fig. 2*B*, lanes *b*, *j*). However, with the introduction of Exo III, the fragments upstream of the 30-bp, resistant site coalesce sharply to a discrete position on its distal edge, no matter which enzyme is used to make the initial cut. The discrete barrier clearly persists even when Exo III activity is increased roughly 100-fold (lane *i*). When the first cut is made by *Xho*I at position −194, Exo III nibbles almost quantitatively from that site to the same discrete stop. The distance travelled by Exo III from the *Xho*I site can be determined precisely (to within 5 bp) by measuring the relative difference in fragment lengths between the two subfragments, using DNA markers in adjacent gel lanes, or mixed in together with the samples (see Fig. 3). Thus the block in the noninduced hsp70 gene is located at position −40.

No Exo III resistance on protein-free DNA

Two potential artefacts can compromise the technique. The discrete block observed could be due to a specific cut made by S_1 nuclease on the purified DNA during the trimming step, or to a peculiar DNA sequence which blocks the passage of the enzyme by virtue of primary or secondary DNA structure alone. In a control experiment using protein-free DNA as substrate (Fig. 2*C*), no specific cuts are shown to be made by S_1 nuclease on free DNA at the position of blockage in chromatin, nor at any other position in the region analysed (lane *c*). Also, no impediments against Exo III digestion due to the DNA sequence alone are observed (lane *d*). Hence, the resistance to Exo III digestion is due to nucleoprotein structure in chromatin.

Heat shock

Does the pattern of Exo III digestion from the *Xho* I cut at −194 change during heat shock? Although the efficiency of restriction is somewhat reduced in the induced hsp70 gene, the digestion pattern is qualitatively different from the noninduced pattern (Fig. 3). Instead of a discrete stop at −40, the enzyme stutters from position −108 to −40, indicating that a comparatively weak barrier (or series of barriers) is encountered, starting at −108. The different patterns of resistance in the normal and the induced genes are found to be similar at both chromosomal loci, using additional restriction cleavage which effectively removes the genes at either locus (data not shown); thus the average pattern is likely to represent each individual hsp70 gene.

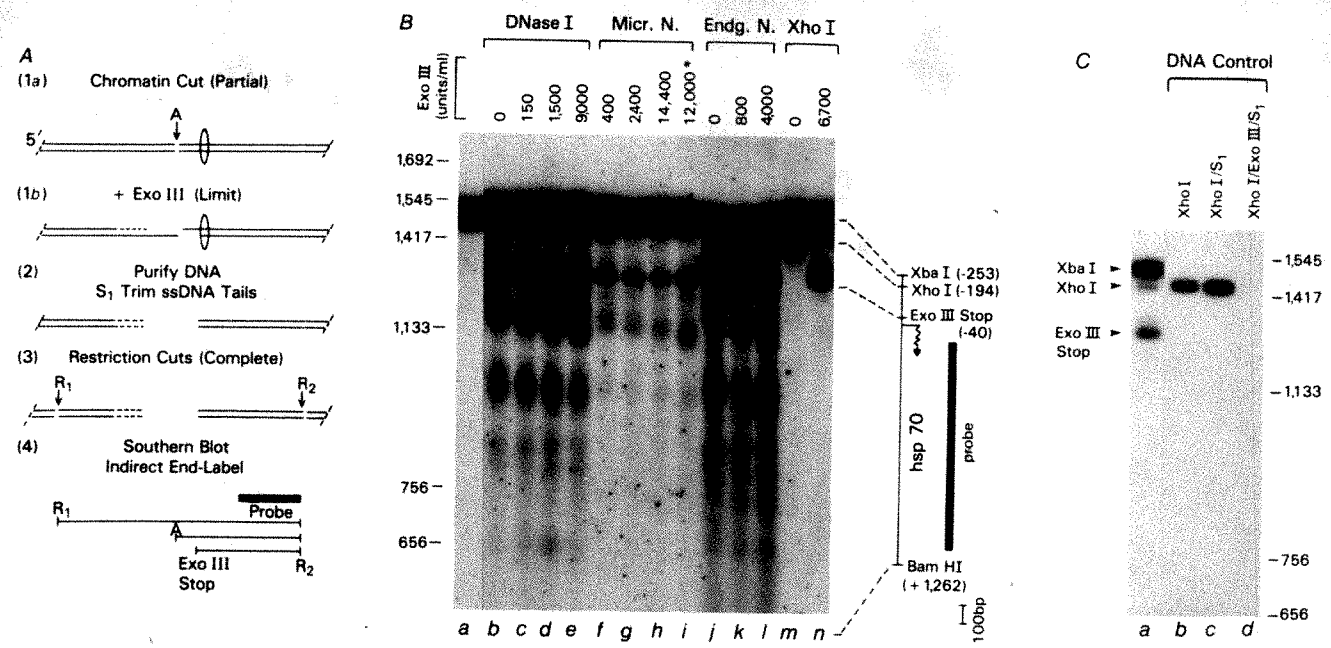


Fig. 2 ***A***, The Exo III protection technique. A hypersensitive site in chromatin is drawn as a region of DNA with a hypothetical protein bound to it. ***B***, Exo III resistance in chromatin in the noninduced hsp70 gene. A partial restriction map of the hsp70 gene is shown[11]. Lane *a*, No enzyme control. SL2 nuclei were mock-incubated in digestion buffer. Lanes *b–e*, SL2 nuclei were digested with DNase I, 10 U ml^{-1} and Exo III for 11 min at 30 °C. Lanes *f–i*, 0–18 h embryonic nuclei were digested with micrococcal nuclease, 9 U ml^{-1}, 3 min, 25 °C. The enzyme was inactivated with EGTA to 0.5 mM, Exo III was introduced and the digestion continued at 30 °C for 7 min (lanes *f–h*) or for 30 min (lane *i*)*. Lanes *j-l*, SL2 nuclei were digested with an endogenous nuclease stimulated by digestion in buffer plus 1 mM $CaCl_2$, at 37 °C for 10 min. $CaCl_2$ was chelated with EGTA to 1.5 mM, Exo III was added and digestion continued at 37 °C for 15 min. Lanes *m*, *n*, SL2 nuclei were digested with *Xho*I at 2,000 U ml^{-1} and Exo III for 10 min, 30 °C. ***C***, S_1 nuclease and Exo III reactions on protein-free DNA.
Methods: ***B***, Isolation of nuclei: SL2 cells were disrupted at 0–4 °C in a Dounce homogenizer in nuclear buffer (60 mM KCl, 15 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EGTA, 15 mM Tris-HCl *p*H 7.4, 0.5 mM dithiothreitol (DTT), 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 0.3 M sucrose). Unbroken cells were pelleted at 1,600*g* for 45 s. The supernatant was centrifuged through 1 vol of nuclear buffer–1.7 M sucrose (stirred interface), at 17,000*g* (average) for 15 min in a swinging bucket rotor. Purified nuclei were resuspended for digestion in nuclear buffer–5% glycerol at 5×10^8 nuclei ml^{-1}. Nuclei from *Drosophila melanogaster* embryos (Oregon R, P2 strain) were prepared after dechorionating in 50% Chlorox, 0.02% Triton X-100 for 3 min at room temperature, by disruption in a Dounce homogenizer in nuclear buffer at 0–4 °C. The homogenate was filtered through a crude paper filter and the filtrate was centrifuged at 1,600*g* for 90 s. The supernatant was centrifuged at 3,700*g* (average) for 5 min in a swinging bucket rotor to pellet crude nuclei. The pellet was resuspended in nuclear buffer, and further centrifuged through nuclear buffer–1.7 M sucrose as above. Purified embryonic nuclei were resuspended for digestion in nuclear buffer–5% glycerol at 1×10^9 nuclei ml^{-1}. Nuclease digestion: Nuclear buffer–5% glycerol was used as the standard buffer for all digestions except those involving micrococcal nuclease, which included 0.4 mM $CaCl_2$. Nuclei were digested with the primary cleavage enzyme plus Exo III (New England Biolabs or Boehringer Mannheim). Digestions were terminated as described previously[5] and nucleic acids were purified by overnight proteinase K digestion, 50 μg ml^{-1}, 37 °C, and by repeated organic extractions followed by ethanol precipitation[6]. S_1 nuclease trimming: Reactions were performed in 40 mM NaOAc, *p*H 4.5, 1 mM $ZnSO_4$ and 250 mM NaCl using 4,000 U ml^{-1} S_1 nuclease (Boehringer Mannheim) at 30 °C for 15 min. The DNA concentration was typically ~0.2 mg ml^{-1}. The reaction was terminated by the addition of EDTA to 5 mM and Tris-HCl *p*H 8.0 to 50 mM. Nucleic acids were purified by organic extraction and ethanol precipitation. Restriction digests and gel electrophoresis: DNA samples (~ 5 μg per lane) were secondarily restricted to completion using *Bam*HI + *Xba*I, together with a trace of RNase A and RNase T1. DNA was electrophoresed on a 40 cm long, 1.4% agarose TAE gel at room temperature for ~35 h. The gel was treated in 0.1 M HCl for 15 min before the DNA was denatured, neutralized and blotted, as described previously[5] except that the blotting solution was 20x SSC, 1 M NH_4OA_c (1x SSC is 0.15 M NaCl, 0.015 M Na citrate *p*H 7.0). The filter was rinsed in 1/2 × blotting solution, dried, baked, prehybridized for 4–8 h at 65 °C and hybridized as described previously[5] without dextran sulphate for 12 h at 65 °C in a slit chamber to a recombinant plasmid labelled with ^{32}P by nick-translation to a specific activity of $\sim4\times10^8$ c.p.m. per μg DNA. The solid bar indicates the region of the hsp70 gene in the plasmid. The filter was washed twice at 65 °C in 2 × SSC, 0.5% SDS and three times in 0.1 × SSC, 0.1% SDS, 65 °C; each wash was for 15 min with agitation. The filter was exposed without intensification. ***C***, 20 μg purified SL2 DNA was restricted with *Xho*I in 25 μl of 100 mM NaCl, 6 mM $MgCl_2$, 10 mM Tris *p*H 7.4. After the reaction 25 μl of nuclear buffer was added and 5 μl of the mixture was removed for secondary *Bam*HI restriction (lane *b*). Lane *c*, DNA was purified by phenol extraction from 22.5 μl mixture. The phenol was removed by ether extraction, the DNA treated with 2,500 U ml^{-1} S_1 nuclease as in Fig. 2*b* legend, purified, and digested with *Bam*HI. Lane *d*, the last 22.5 μl of mixture were digested with 4,000 U ml^{-1} Exo III at 30 °C for 10 min. DNA was purified, S_1 trimmed, repurified and restricted with *Bam*HI. Samples *b–d* were electrophoresed, blotted and hybridized to hsp70 probe as in *B* legend, along with a typical *Xho*I + Exo III reaction on chromatin for comparison (lane *a*).

Other side of barrier

The other side of the barrier to Exo III in the normal and induced hsp70 genes is mapped using *Pvu*II to make the initial cut; the enzyme cleaves at position +65, inside the proximal boundary of the DNase I hypersensitive site (Fig. 4). The hybridization probe labels the 3.56-kb fragment containing one hsp70 gene, but is also weakly homologous to other *Bgl*II fragments; these do not obscure the analysis of the hsp70 subfragments. In both normal and induced samples, a discrete barrier which maps to position −12 is observed (Fig. 4, lanes *c, d, f, g*). A control experiment using protein-free DNA as substrate indicates that this barrier is due neither to artefactual S_1 cleavage nor to the DNA sequence alone (data not shown).

An endogenous exonuclease activity is also observed here, in the reactions without Exo III, but this activity is blocked at essentially the same position (Fig. 4, lanes *b, e*). Taking into account the uneven amounts of DNA loaded, proportionately more of the *Bgl*II–*Pvu*II fragment is chased into the subfragment, by addition of Exo III. The endogenous activity nibbles predominantly in a single-stranded manner since *Pvu*II restriction of chromatin without subsequent S_1 nuclease reaction gives a fragment which co-migrates with a whole *Bgl*II-*Pvu*II fragment (lane *a*). As expected from previous work[27], the sensitivity of a *Pvu*II site at +1,034 in the hsp70-coding region is increased during induction, but no discrete subfragments due to exonuclease activity emerge from this site.

In summary, convergent Exo III digestion from cuts on each flank of the DNase I hypersensitive site reveals strong blockage at positions −40 and −12 respectively, and delimits a 28-bp, resistant site (site I) for the noninduced hsp70 gene. This agrees well with the earlier mapping of the 30-bp, DNase I resistant site from −38 to −8. For the induced hsp70 gene, strong blockage again at −12 suggests retention of site I. In addition, the diffuse blocks from −108 to −40 reveal a new site of weaker resistance (site II), the distal border of which lies at −108; its proximal border cannot be mapped from the other side because the enzyme is unable to penetrate site I.

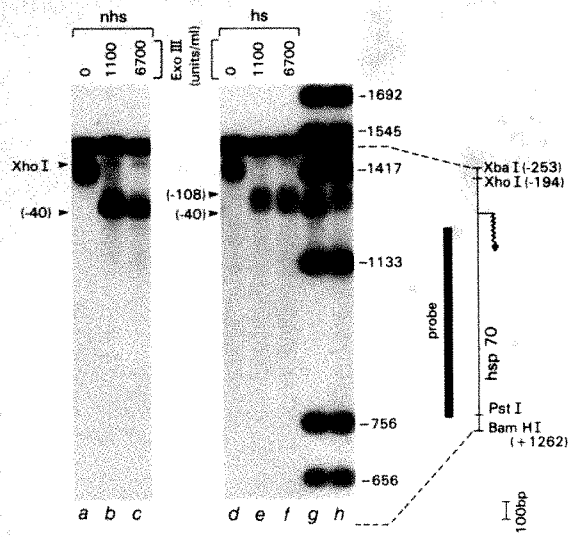


Fig. 3 Exo III digestion from the *Xho*I cut at position −194 in normal and induced hsp70 gene chromatin. Isolated nuclei from 0–18 h old, nonshocked embryos (lanes *a-c*) and from embryos heat-shocked for 15 min at 36 °C (lanes *d–f*), were digested for 10 min at 30 °C with *Xho*I at 2,000 U ml^{-1} plus Exo III as indicated. Purified DNA samples were digested with S_1 nuclease, restricted secondarily with *Bam*HI + *Xba*I, electrophoresed, blotted and hybridized with the hsp70 gene probe as described in Fig. 2*B* legend. The Exo III stop positions in base pairs upstream from the mRNA start (+1) are indicated beside the arrowheads. Lanes *g* and *h* are the same samples as in *b* and *e* spiked with pBR322 marker fragments, ~60 pg each (pBR322 restricted with *Bam*/*Pvu*II; *Bam*/*Pst*; EcoRI/*Sal*I/*Pvu*II/*Pst*I).

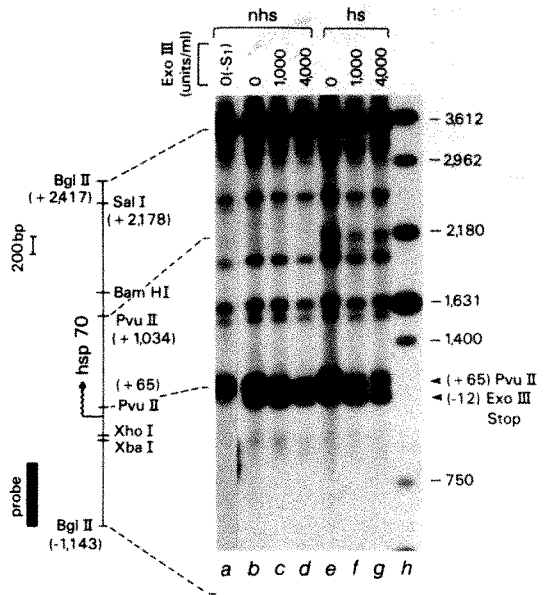


Fig. 4 Exo III digestion from the *Pvu*II cut at position +65 in normal and induced hsp70 gene chromatin. A partial restriction map of the first of the two hsp70 genes in tandem at chromosomal locus 87C is shown[11]. Isolated nuclei from nonshocked SL2 cells (lanes *b–d*) and from cells heat shocked for 15 min at 36 °C (lanes *e–g*) were digested for 40 min at 37 °C with 600 U ml^{-1} *Pvu*II plus Exo III as indicated. DNA was purified, trimmed with S_1 nuclease, repurified, restricted with *Bgl*II, electrophoresed on a 30 cm 1.2% agarose gel, blotted and hybridized with the cloned probe (solid bar) as described in Fig. 2*B* legend. An arrowhead indicates the Exo III (and endogenous nuclease) stop position. Similar results are obtained using normal and heat-shocked embryos. Lane *a* is a *Pvu*II digest of nonshocked SL2 nuclei at 400 U ml^{-1}, 30 °C, 20 min; the purified DNA was restricted with *Bgl*II without prior S_1-nuclease treatment, and processed as above. Lane *h* contains pBR322 markers as in ref. 5.

Exo III resistance on hsp83 gene

The single-copy gene encoding hsp83 resides at chromosomal locus 63BC and consists of a 3.7-kb transcription unit containing two exons separated by a 1.1-kb intron[28–31]. I have previously mapped a cluster of three DNase I hypersensitive sites, one of which encompasses the 5′ terminus of the hsp83 gene[5]. The Exo III digestion pattern from a *Eco*RI cut at position −166 is shown in Fig. 5*A*. In noninduced *Drosophila* embryos, the principal subfragment among the heterogenous collection produced by the endogenous exonuclease and by Exo III locates a barrier at position −39 (Fig. 5*A*, lanes *b*, *c*). On heat induction, this barrier shifts sharply to position −86 (lanes *d*, *e*).

The other side of the barriers are located by using exonuclease to nibble upstream from a *Rsa*I cut at position +20 (Fig. 5*B*). In noninduced *Drosophila* embryos, the principal subfragment produced by the endogenous exonuclease locates a weak barrier at position −17 (Fig. 5*B*, lanes *b*, *c*). Addition of Exo III further degrades that subfragment; there is a minor pause at −61 (lanes d, e). In heat-shocked embryos, two sharply discrete subfragments, one chasing into the other, are produced by the endogenous exonuclease and by Exo III (Fig. 5*B*, lanes *f*, *g*, *h*, *i*), locating two barriers at positions −17 and −50 respectively. No resistant sites are found in the controls using free DNA (data not shown). The relatively lower yield of cleaved subfragments in noninduced samples in Fig. 5 is primarily due to a less efficient initial restriction cut.

In summary, the resistance to convergent exonuclease digestion at −39 and −17 delimits a relatively weak, 22-bp resistant site (site I) for the noninduced hsp83 gene. For the induced gene, blockage again at −17 suggests the retention of site I. In addition, a 36-bp resistant site from −86 to −50 is revealed (site II). The distal border of site I cannot be mapped here because exonuclease is unable to penetrate site II. No highly resistant sites are found within the other two DNase I hypersensitive sites clustered near the 5′ end of the hsp83 gene (data not shown).

TATA and upstream sequences

The resistant sites are aligned over the nucleotide sequences[29] upstream of the hsp83 and hsp70 transcriptional starts in Fig. 6*A*. They lie within the 5′ flanking region involved in the regulation of these genes[32–36]. Strikingly, for both hsp83 and hsp70 genes, site I surrounds the TATA box sequence which is indispensable for accurate transcriptional starts[37]. For the induced hsp83 gene, site II covers the upstream consensus sequence CTNGAANNTTCNAG that is required for heat-inducible expression in heterologous transient assays[33,35]. This upstream element is also contained within the more diffuse site II in the induced hsp70 gene, although the site does extend an additional 45 bp upstream. Curiously, site I is strong in the hsp70 gene whereas site II is strong in the hsp83 gene.

Site II does directly cover the consensus sequence in the induced hsp83 gene. Before induction, two *Xba*I sites at −74 and −64, essentially coincident with the upstream element, are hypersensitive to *Xba*I cleavage in chromatin when compared with a site at +484 within the hsp83 transcription unit. After induction, the upstream *Xba*I sites are almost totally resistant, whereas the latter is more sensitive (Fig. 5*B*, lanes *j*, *k*). A rough analysis (not shown) indicates that sites I and II in the hsp83 gene are also resistant to DNase I digestion.

Discussion

The resistant sites surrounding the TATA box sequence and the upstream control element cover 22 bp or 28 bp and 36 or <68 bp respectively, depending on the gene. This range is considerably less than the 146 bp of DNA resistant to enzyme

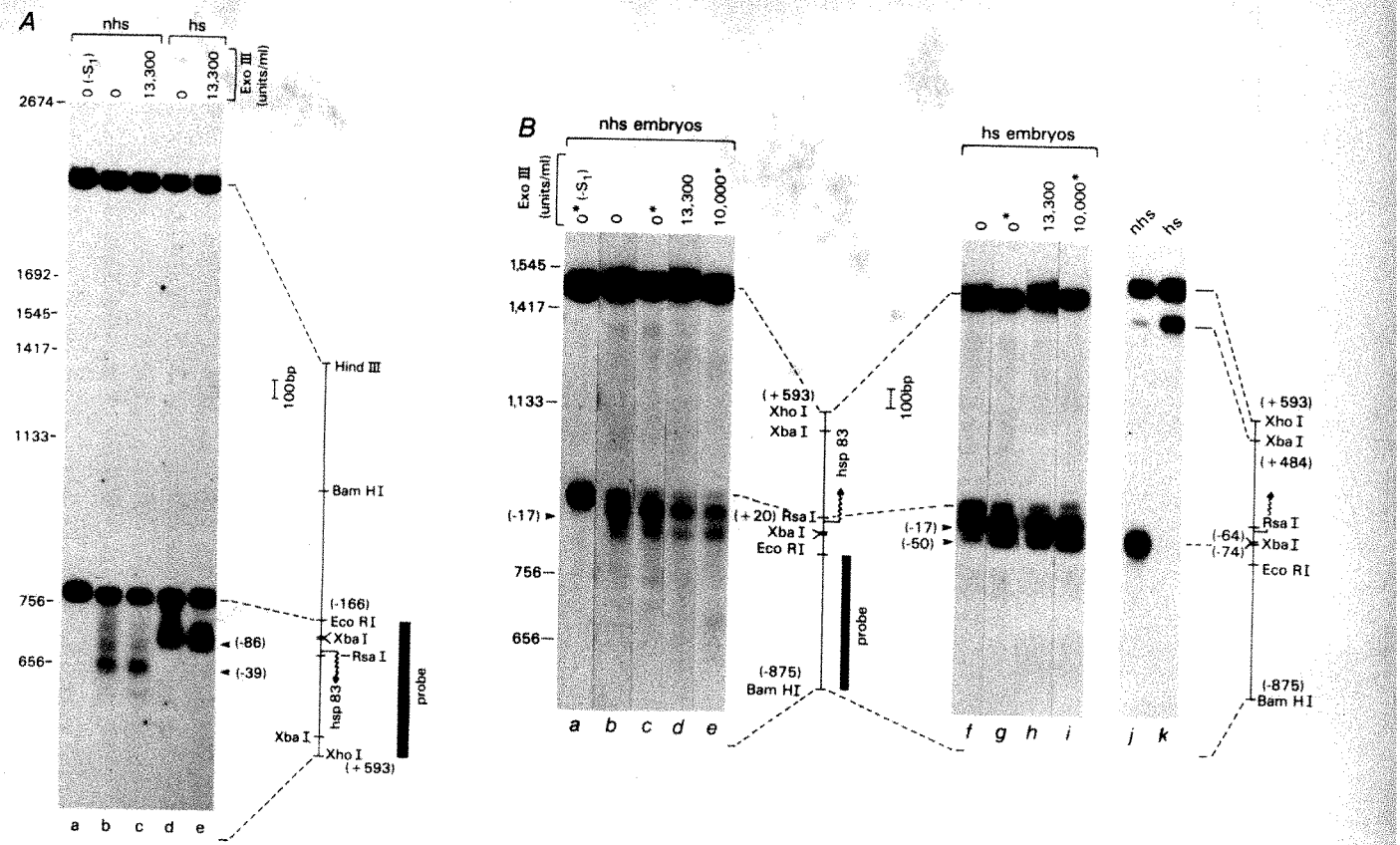


Fig. 5 ***A***, Exo III digestion from the *Eco*RI cut at position −166 in normal and induced hsp83 gene chromatin. A partial restriction map of the hsp83 gene region is shown[28,30]. Isolated nuclei from normal (lanes *a-c*) and heat-shocked (lanes *d, e*) 2–8 h-old embryos were digested with *Eco*RI at 4,200 U ml^{-1} plus Exo III as indicated for 7 min at 30 °C. The purified DNA was trimmed with S_1 nuclease (except in lane *a*), repurified, restricted with *Xho*I + *Hind*III, electrophoresed on a 40-cm long, 1.4% 50 mM Tris-borate EDTA *p*H 8.3 (TBE) gel, blotted and hybridized with the cloned probe (solid bar) as described in Fig. 2*B* legend. Arrowheads indicate the stop positions of Exo III and the endogenous exonuclease. ***B***, Exo III digestion from the *Rsa*I cut at position +20 in normal and induced hsp83 gene chromatin. Isolated nuclei from normal (lanes *a–e*) and heat-shocked (lanes *f–i*) 2–8-h-old embryos were digested with 1,500 U ml^{-1} *Rsa*I plus Exo III as indicated, for 15 min at 35 °C (lanes *a, c, e, g, i*)* or with 1,700 U ml^{-1} *Rsa*I plus Exo III as indicated for 7 min at 30 °C (lanes *b, d, f, h*). The purified DNA was trimmed with S_1 nuclease (except in lane *a*), repurified, restricted with *Bam*HI + *Xho*I, electrophoresed on a 1.4% 50 mM TBE gel, blotted and hybridized with the cloned probe (solid bar) as described in Fig. 2*B* legend. Lanes *j, k*, *Xba*I cleavage of hsp 83 chromatin. Isolated nuclei from normal (lane *j*) and heat-shocked (lane *k*) 2–8-hr-old embryos were digested with 1,400 U ml^{-1} *Xba*I at 35 °C for 15 min. The purified DNA was restricted with *Bam*HI + *Xho*I, electrophoresed beside the above samples and processed similarly.

digestion in the nucleosome core particle[38–40], but is similar in size to the fragments resistant to digestion when prokaryotic activator and repressor proteins are bound to DNA[41]. We suggest that the resistance is due to at least two nonhistone proteins which bind specifically to duplex DNA in the TATA box region (TATA-binding protein(s), TAB), and the upstream heat-shock control region (heat-shock activator protein(s), HAP) respectively (Fig. 6*b*). (Since Exo III is also unable to digest single-stranded DNA, the resistance to cleavage could conceivably occur by a melting of the sequences, but this is improbable because the sites are resistant to DNase I cleavage too.)

We conjecture that the binding of TAB establishes the transcriptional potential of heat-shock genes in (post-blastoderm) embryos. This potential is realized during heat-shock activation by the additional binding of HAP. The fact that site II only appears in the active gene argues that HAP is a positive activator of transcription; a repressor should have had the reverse effect. Others have also argued for positive regulation in the sense that deletions in the upstream element reduce transcription during heat shock rather than cause constitutive expression[33,34]. Only after TAB and HAP are bound might transcription be initiated by RNA polymerase II. The significance of protein-protein contacts in the binding of polymerase II to eukaryotic promoters has been argued[42].

The displacement of a nucleosome from the TATA box region by TAB, combined with the perturbation of higher order contacts between neighbouring nucleosomes in the native chromatin fibre, could be sufficient to create nuclease hypersensitivity over the adjacent sequences, its relative intensity and spread being influenced by the local sequence order. Such a displacement could occur during transient reassembly of chromatin during DNA replication.

Is the TATA box sequence in all genes which possess it bound to TAB in chromatin? This seems unlikely in view of other work on developmentally regulated genes which suggest a nucleosomal arrangement over promoter sequences in all but the expressing tissue. Although no obvious additional homologies have been generally found within 10 bp around the TATA box[37], Holmgren *et al.*[29] have pointed out an eight base homology (A/G)C(C/A)GGCGC immediately following the TATA box in the heat-shock gene family, which, curiously enough, is also present at the same locations in the *Drosophila* histone H4 and alcohol dehydrogenase genes (noted in ref. 33) but not in other *Drosophila* genes. All TATA binding functions could be governed by a small group of TAB proteins, each of which is able to bind to a 'TATA(+)' consensus sequence common to one or more gene families. Davidson *et al.*[43] have recently reported a Hela cell factor which is necessary for *in vitro* transcription from conalbumin and adenovirus late promoters, which binds to the TATA box region in the absence of polymerase II and forms a stable pre-initiation complex. It is interesting to speculate that TAB may fall into this class of transcription factors.

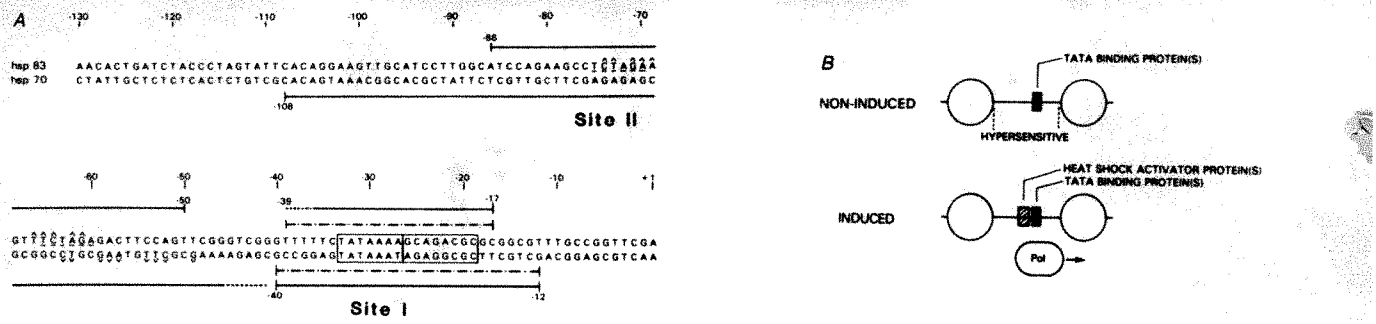


Fig. 6 ***A***, Exonuclease resistant sites aligned over nucleotide sequences upstream of the 5′ ends of the hsp83 and hsp70 genes. The sequences are copied from Holmgren *et al.*[29]. The initiation site of transcription of both genes is shown as +1. Boxed sequences indicate homologies shared between these and other *Drosophila* heat-shock genes[29]. Capped nucleotides indicate the consensus sequence that is required for heat induction[35]. Underlined nucleotides indicate the two *Xba*I sites upstream of the hsp83 gene. Lines above and below the sequences are resistant nucleotides in hsp83 and hsp70 gene chromatin respectively. —·—·—, Resistant nucleotides in the noninduced genes; ——, resistant nucleotides in the induced genes. Boundaries of the resistant regions are precise to five base pairs. Undeterminable boundaries are indicated by dots following the solid lines. ***B***, A model for the heat-shock promoter region in chromatin during normal and induced states. Circles represent nucleosomes; their positions have not been determined. Pol refers to RNA polymerase II.

Our simple model does not attempt to account for the more subtle nuances of heat-shock gene expression, such as the developmental control which is superimposed on the hsp83, hsp28 and hsp26 genes in *Drosophila* ovarian nurse cells[44]. We hope it will be useful as a framework for comparison with the rest of the heat-shock gene family and for defining the minimal components essential for heat-shock gene expression. Aspects of the model are generally applicable to the regulation of other eukaryotic genes.

This work has focused on the localization of barriers against convergent Exo III nibbling from cuts on either side of a nuclease hypersensitive site. Divergent cleavage, presumably into adjacent nucleosomal DNA, might reveal the positions of neighbouring nucleosome core particles. We have not extended our analysis into this area, except in one example averaged over the five heat-shock genes, and this result appears to argue in favour of a statistical[45] location of these nucleosomes (Fig. 2*B*). A more detailed analysis is required to address this question properly.

The Exo III protection technique could be used for mapping other protein-binding sites in chromatin. Clean results are obtained especially when a single-cut restriction enzyme is used to make the initial cleavage in the hypersensitive region. We have found empirically that mild conditions for restriction and Exo III cleavage (30 °C, 10–15 min) using several thousand units per ml of restriction enzyme can result in cleavage of more than 50% of the molecules at a particular hypersensitive site, of which roughly 50–100%, depending on the site, can be digested further by the endogenous exonuclease or Exo III. Thus, the resistant sites occur in a significant fraction of the overall population. For ultimate precision in site mapping, the exonuclease resistant fragments can be separated on sequencing gels (without prior S_1 trimming), and the appropriate strand labelled with a single-stranded probe after blotting to nylon membranes according to a new protocol[46].

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Two types of binary radio pulsars with different evolutionary histories

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The four known binary radio pulsars (Table 1) seem to fall into two different categories. Two of them, PSR0655+64 and PSR1913+16, have short orbital periods (<25 h)[1,2] and high mass functions, indicating companion masses >0.8 $M_\odot$ (respectively, about 1 $M_\odot$ and 1.4 $M_\odot$ (ref. 2)). The other two, PSR0820+02 and PSR1953+29, have long orbital periods (≳ 120 days), nearly circular orbits and low almost identical mass functions of ~3×10^{-3} $M_\odot$, suggesting companion masses of ~0.2–0.4 $M_\odot$ (refs 3–5). We point out here that these two classes of systems are expected to be formed by the later evolution of binaries consisting of a neutron star and a normal companion star, in which the companion was (considerably) more massive than the neutron star, or less massive than the neutron star, respectively. Mass transfer from an evolved companion that is more massive than the neutron star (more precisely, mass ratio ≳0.85 (refs 6, 7)) tends to be unstable and to lead to runaway mass transfer and spiral-in resulting in a very short orbital period, whereas mass transfer from an evolved companion that is less massive than the neutron star is stable and leads to expansion of the orbit[6,7]. Furthermore, we point out that in the systems with a less massive companion, the neutron star most probably was formed by the accretion-induced collapse of a white dwarf. Such a model explains in a natural way why PSR1953+29 has a millisecond rotation period and why PSR0820+02 has not.

When the companion has a mass several times that of the neutron star (≳3–4 $M_\odot$, see below) and the system is relatively wide (orbital period ≳ a few weeks) so that at the onset of the mass transfer the companion has a deep convective envelope, runaway mass transfer is unavoidable. This is because (1) mass transfer from the more massive to the less massive star leads to shrinkage of the Roche lobe of the more massive star, while (2) a convective envelope tends to expand when the star loses mass[8]. This will, on a time scale of ~10^3 yr, lead to the formation of an extended convective common envelope in which the neutron star and the evolved core of the companion will spiral towards each other as a consequence of the large frictional drag[9–12]. The duration of this spiral-in process, in which finally the envelope is lost, is expected to be short, ~10^3–10^5 yr (refs 11–13).

The final result is expected to be a system consisting of the neutron star and the evolved core of the companion with an orbital period ranging from ~1 h to a few days (in analogy to the case of cataclysmic variables which are products of a similar type of spiral-in evolution[9,12]).

In order to have deep spiral-in, the two components should differ sufficiently in mass, preferably by more than a factor of two, because otherwise soon after the onset of the mass transfer the mass ratio will be reversed which would lead to subsequent spiral-out. (For systems with mass ratios in the range 0.85–2 the outcome of the evolution is more complex and, for the sake of argument, will not be considered here.)

Figure 1 depicts as an example the anticipated evolution of a binary initially consisting of a $M = 5M_\odot$ star and a neutron star, with an orbital period of ≳80 days. The $5M_\odot$ star overflows its Roche lobe when it has exhausted helium in its core (that is, when it climbs up along the asymptotic giant branch, AGB). At helium exhaustion it has a 0.95 $M_\odot$ degenerate CO core[14] surrounded by He and H-burning shells. During its ascent of the AGB this core mass increases to 1.39 $M_\odot$. We assume that the star engulfs its companion when $M_{core} \simeq 1.0\ M_\odot$, implying a binary period of about 100 days (see Table 2). The system after spiral-in will consist of a 1.0 $M_\odot$ CO white dwarf and a neutron star with a narrow and a circular orbit, that is, closely resembling the PSR0655+64 system (see Table 1). The type of binary evolution considered here is the so-called case C [6,7]. (In shorter-period systems—case B—the evolution will be somewhat more complex and may lead to several stages of mass transfer[15,16]; the outcome will, however, in most cases be roughly similar[15].) Table 2 lists the maximum and minimum orbital periods for case C evolution for companion masses in the range 3–7 $M_\odot$, derived from Paczynski's[14] evolutionary tracks. The corresponding CO core masses are also listed.

Table 2 shows that an evolution similar to the one depicted in Fig. 1 is expected in all wide (case C) neutron star binaries in which the mass of the companion is in the range ~5 to ~8 $M_\odot$, as on the AGB all such stars develop degenerate CO cores with a mass in the range 0.95–1.39 $M_\odot$ (refs 14, 17, 18). For companion masses of 3–5 $M_\odot$, the same holds if the binary periods are ≳80 days, such that after helium exhaustion the core can still grow to ≳0.90 $M_\odot$ before the star overflows its critical lobe. Companions more massive than ~8–10 $M_\odot$ will develop evolved cores too massive to terminate as white dwarfs[15,19]. Following the spiral-in and subsequent nuclear evolution such a core is expected to collapse to a neutron star. The mass ejection in this second supernova in the system may induce a considerable orbital eccentricity or may even disrupt the system[20,21]. PSR1913+16 is expected to have been formed in this way[21,22] (in the case of disruption, two runaway neutron stars will be formed, one young and one old).

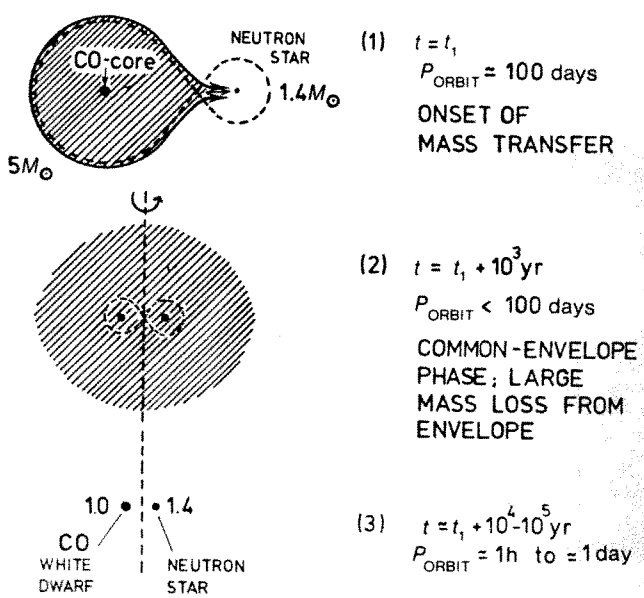


Fig. 1 Anticipated evolution of a relatively wide binary consisting of a 5 $M_\odot$ star with a neutron star companion. At the onset of the mass transfer the 5 $M_\odot$ star is a giant with a 1 $M_\odot$ degenerate CO core and a gradually expanding envelope (1). Rapidly, a common convective envelope forms (2) in which the CO-core and the neutron star spiral-in. During this spiral-in the envelope is ejected due to frictional heating and a very close system remains (3) consisting of a neutron star and a massive CO-white dwarf in a circular orbit. All wide (case C) neutron star binaries with companions in the range 3 $M_\odot$ to 8–10 $M_\odot$ are expected to evolve in this way. For companions more massive than 8–10 $M_\odot$ the evolved core after spiral-in collapses to a neutron star, and a close neutron star binary with an eccentric orbit (or two runaway pulsars) will be formed[20–22].

At the onset of the spiral-in the neutron star will often be older than 10^7 yr (companion stars of 5–15 $M_\odot$ need some (1–5) × 10^7 yr to leave the main sequence) such that its surface magnetic field strength may have decayed by several orders of magnitude. (The space distribution of radio pulsars with known proper motions indicates that the dipole strength of the surface magnetic field of a neutron star decays on a time scale of (2–5) × 10^6 yr (refs 23–25).) With a canonical magnetic dipole strength at birth of $\sim 10^{12-13}$ G (refs 23–25), the dipole strength at the onset of spiral-in may thus be $\sim 10^{10-11}$ G, as observed in PSR0655+64 and PSR1913+16 (see Table 1). During the spiral-in the accretion onto the neutron star will take place near the Eddington-limited rate $\dot{M}_{\rm Edd}$, implying that its rotation will be spun-up to a minimum period that depends practically only on the surface dipole strength B_9 (in units of 10^9 G) as[21,26,27]

$$P = (2.7\ \text{ms})\ B_9^{6/7}\ R_6^{15/7} \left\{\frac{M}{1.4\ M_\odot}\right\}^{-5/7} \left\{\frac{\dot{M}_{\rm Edd}}{\dot{M}}\right\}^{3/7} \qquad (1)$$

where R_6 is the neutron star radius in units of 10^6 cm and M is its mass. After the loss of the common envelope these neutron stars will become observable as radio pulsars with a relatively low surface dipole magnetic field strength[21,22,24]. For the observed B_s values of PSR0655+64 and PSR1913+16 (Table 1), and $R_6 = 1$, $M = 1.4\ M_\odot$, equation (1) yields $P = 0.17$ and 0.04 s, respectively, in reasonable agreement with their observed pulse periods (allowing for some spin-down since their formation[21,24]).

The absence of radio pulses from the second (younger) neutron star in the PSR1913+16 system may be due either to beaming effects, or to the relatively short spin-down time scale of a newborn strong-magnetic field neutron star (if the second supernova in the system took place a few million years ago, the new neutron star may already have reached the turn-off period of a few seconds whereas the 'recycled' old one, due to its weaker surface dipole field, will remain observable as a pulsar for a much longer time[21,22,27,28]). The best known progenitor candidates for the above described type of evolution seem to be the B-emission X-ray binaries, which have orbital periods ranging from 15 days to several years[29,30], and companion masses ranging from $\sim 7\ M_\odot$ (spectral type B3Ve) to $\sim 20\ M_\odot$ (spectral type O9Ve). The companion of PSR0655+64 should have had a mass below the limit for exploding as a supernova, $\leq$8–10 $M_\odot$ (in case C; in case B: $\leq$10–12 $M_\odot$) whereas that of PSR1913+16 had a mass above this limit (see ref. 21 for details).

We now turn to the alternative case in which the system started out with a companion star less massive than the neutron star. When in this system (mass ratio ≤ 0.85) the companion begins to overflow its Roche lobe, the ensuing mass transfer will be self-stabilizing as it leads to expansion of the orbit. Webbink *et al.*[31] and Taam[32] have shown that in this way, starting with companions with $M \leq 1.2\ M_\odot$ and orbital periods upwards from about 1 day, one obtains a long-lasting ($\sim 10^6$–10^8 yr) stage of relatively high mass transfer ($\dot{M} \geq 10^{-8}\ M_\odot\ \text{yr}^{-1}$).

During this phase the companion ascends the giant branch and has a degenerate helium core with a mass in the range 0.2–0.45 $M_\odot$, and generates energy by burning hydrogen in a shell around this core. The mass transfer, and the associated expansion of the orbit, are driven by the gradual growth of the core mass. The duration of the mass transfer phase depends mainly on the initial mass of the companion and the initial orbital period. In the end, only the degenerate helium core of the companion is left behind, as a helium white dwarf, and the final orbit will always be wide, as the mass and orbital angular momentum of the system are, in first approximation, expected to be conserved.

Savonije[33], Paczynski[34] and Joss and Rappaport[35] have, independently, pointed out that the final systems resulting from this evolution show a striking resemblance to that of the 6-ms binary radio pulsar PSR1953+29, as (1) the final companion mass will be low ($\sim 0.3\ M_\odot$) and (2) the orbit will be (almost) circular as a consequence of the long-lasting preceding mass transfer stage. (When the companion is a red giant which is filling its Roche lobe, tidal dissipation in its convective envelope will be very efficient[36,37].) Rappaport and Joss[35] showed that for an initial mass of the companion of 1 $M_\odot$, the most likely initial orbital period of PSR1953+29 was around 10 days and that of PSR0820+02 about 1 yr. The duration of the mass-transfer phase in these systems was (3.5–7.7) × 10^7 yr and 4 × 10^6 yr, respectively (similar results are obtained for a companion mass of 1.2 $M_\odot$, as can be easily verified).

The above outlined evolutionary model for these wide systems thus seems to explain their observed orbital characteristics, including their mass functions; but this model at the same time implies that the systems must be very old (at least several times 10^9 yr) as the companion stars should have started out with masses $\leq 1.2\ M_\odot$ and had already completed their main-sequence evolution before the onset of the mass transfer. Because surface dipole components of magnetic fields of neutron stars seem to decay on a time scale $\leq 10^7$ yr one would, therefore, not expect any detectable surface dipole fields to be left in them.

However, PSR0820+02 and PSR1953+29 still have a surface dipole magnetic field strength of 3 × 10^{11} G and $> 10^9$ G, respectively (Table 1). The same problem exists in the GX 1+4 system which is an 120 s X-ray pulsar, and must be a very wide system as the companion star is an M6IIIe red giant[38]. Such a system seems an excellent progenitor candidate for a system like PSR0820+02 (ref. 39). From its spin-up rate in combination with its X-ray luminosity of $\sim 10^{38}$ erg s^{-1} (ref. 38) one derives a lower limit to its magnetic field strength of $\sim 3 \times 10^{11}$ G (ref. 26), which is similar to that of PSR0820+02. Because an M6IIIe giant cannot be more massive than $\sim 3\ M_\odot$ (ref. 14), the age of this system is at least $\geq 2 \times 10^8$ yr (the main-sequence lifetime of a 3 $M_\odot$ star). Moreover, the position close to the galactic centre and at high z suggests an old-disk population old age of (5–10) × 10^{10} yr.

The only consistent way in which one may explain why the neutron stars in these old (wide) systems still can have such fairly strong surface magnetic fields seems to be if they were formed only recently—during the mass-transfer stage itself, by the accretion-induced collapse of a (relatively massive) white dwarf. (It seems reasonable to assume that, regardless of the formation mechanism, all neutron stars are formed with a canonical surface dipole magnetic field strength of 10^{12-13} G, due to dynamo action during the collapse process[25].) Because in the case of PSR1953+29 the mass transfer stage lasted (3.5–7.7) × 10^7 yr and for PSR0820+02 it lasted only $\sim 4 \times 10^6$ yr, the time elapsed since the collapse in these systems may be relatively short: a few times 10^7 yr in the case of PSR1953+29 and $< 10^7$ yr in the case of PSR0820+02, respectively. Hence, one would not expect their surface magnetic fields to have decayed by more

Table 1 Orbital and pulse properties of the four binary radio pulsars together with estimates of their surface magnetic field strengths and the masses of their companions

Name	$P_{\rm orb}$ (day)	e	Mass function ($M_\odot$)	Most likely companion mass ($M_\odot$)	$P_{\rm pulse}$ (s)	B_s (G)	Ref.
PSR1913+16	0.32	0.617	0.1322	1.40 ± 0.05	0.059	2×10^{10}	1
PSR0655+64	1.03	0.000	0.0712	1.00 ± 0.30	0.196	8.6×10^{10}	2
PSR0820+02	1,232	0.012	0.00301	0.2 – 0.4	0.865	3.3×10^{11}	4
PSR1953+29	~120	<0.05	0.00272	0.2 – 0.4	0.006	$\sim 2 \times 10^9$ (predicted)	5, 47

Table 2 Minimum and maximum orbital periods for neutron star binaries in which the companion overflows its critical lobe after it has exhausted helium in its core (case C evolution)

Companion mass	P_{min} (day)	M_{core} (He exhaustion) ($M_\odot$)	P_{max} (yr)	M_{core} (max) ($M_\odot$)
$3M_\odot$	32	0.51	5.5 (19)	1.39
$5M_\odot$	76	0.95	4.5 (13)	1.39
$7M_\odot$	173	1.02	3.6 (10)	1.39

A neutron star mass of 1.40 $M_\odot$ was assumed and the corresponding masses M_{core} of the degenerate CO-core of the companion are indicated (Paczynski's[14] evolutionary tracks were used). The first value of P_{max} corresponds to the moment at which the orbital separation equals the radius of the companion; the value within parentheses corresponds to the moment at which the companion fills its Roche lobe.

than a factor of 10^3 and 10^1, respectively. That PSR0820+02 indeed still has the strongest magnetic field of the two is therefore in excellent agreement with the prediction of this accretion-induced collapse model.

In addition, the conditions for achieving accretion-induced collapse are expected to be favourable just in systems with a low-mass ($\leq 1.2\ M_\odot$) giant component for the following reasons. To reach collapse the white dwarf (consisting of CO or O–Ne–Mg) should be able to grow substantially by accretion—as the *a priori* probability that it was born with a mass close to the Chandrasekhar limit will be very low. Just at the accretion rates produced by low-mass giant companions (in the range $10^{-8}\ M_\odot\ yr^{-1}$ to $2\times10^{-7}\ M_\odot\ yr^{-1}$) a considerable growth of the white dwarf is indeed possible as the accreted hydrogen burns in shell flashes that are too weak to cause mass ejection[40–42]. At lower accretion rates, $<10^{-8}\ M_\odot\ yr^{-1}$, hydrogen burns with very strong nova-like flashes in which part (and possibly all) of the accreted matter is ejected such that a substantial growth of the white dwarf is unlikely. In addition, at the age of these systems ($\geq 10^9$ yr) the oxygen in a carbon–oxygen white dwarf may have separated from the carbon and have settled in the core[43], creating a situation favourable for the occurrence of accretion-induced collapse[44].

Because, at any time during the accretion phase the envelope mass of the white dwarf is small (more than a few hundredths of a solar mass of hydrogen would not fit inside its Roche lobe) and because the mass loss of the white dwarf during the collapse need not exceed the equivalent of the binding energy of the neutron star ($\sim 0.1\ M_\odot$), the orbital eccentricity induced by the mass loss in the collapse need not exceed 0.05 (ref. 20) and the orbit may be subsequently circularized by the continued mass transfer from the companion and by tidal forces[39,45].

The above described model is entirely consistent with the very short (6 ms) pulse period of PSR1953+29, because in the PSR1953+29 system the collapse can have occurred several times 10^7 yr before the end of the mass-transfer phase. Consequently, the surface dipole field had time to decay to $\leq 2\times10^9$ G and according to equation (1), spin-up to $\sim$5 ms was possible. On the other hand, in view of the much shorter mass-transfer time scale in the PSR0820+02 system, such a field decay and spin-up to a short period were not possible here.

Thus we conclude, on the basis of the above arguments, that the existence of the two binary radio pulsars with wide circular orbits and low-mass functions, provides the first very strong evidence that neutron stars can be formed in an old stellar population by the accretion-induced collapse of a white dwarf in a relatively wide binary (Whelan–Iben model[46]).

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Cosmic dust collection by the capture cell technique on the Space Shuttle

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The capture cell technique is a new approach to the study of extraterrestrial material: although complementary to stratospheric collection techniques[1], it avoids selection effects introduced by the deceleration of dust in the Earth's atmosphere. The method, to be used extensively in cosmic dust retrieval experiments on the forthcoming NASA Long Duration Exposure Facility, now yields results from its first application in the microabrasion foil experiment (MFE) which was flown as part of the scientific payload on the STS-3 Space Shuttle mission. Post-flight analysis yielded four hypervelocity impact perforation events during 8 days of exposure to space, and confirmed the capture cell technique as a viable low-cost method for the assessment of the near-Earth particulate environment. The effectiveness of a meteoroid bumper[2] in dissipating incident particle energy is strikingly demonstrated; markedly different and yet unexplained perforation morphologies are observed and a revision of the current near-Earth microparticle influx model[3] is suggested.

MFE was exposed over 8 days in a circular Earth orbit of 241 km altitude and 38° inclination, recording a total of one large (23 μm diameter) and three near-marginal perforations

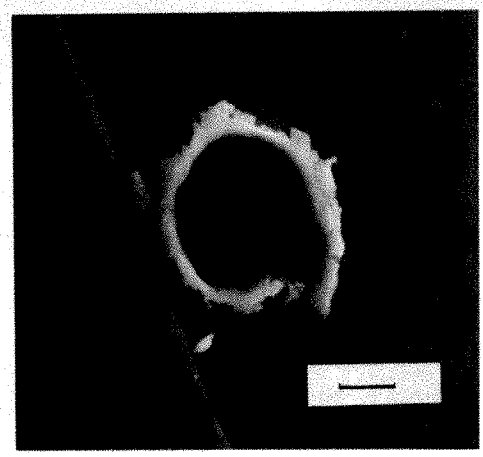


Fig. 1 Scanning electron micrograph of the impact side of the largest aluminium foil perforation (23 μm diameter). Evidence of target melting, raised crater lips and circular symmetry, and especially close correspondence with the exit side (see Fig. 2*a*) indicates that it was caused by a particle considerably smaller than 23 μm; at an assumed velocity of 15 km s^{-1}, a particle diameter of 10 μm is required based on simulation and experiment. Scale bar, 10 μm.

(5.1, 6.5 and 7.5 μm in diameter, respectively). The experiment comprises a double layer foil structure[4]; the top layer of 5-μm aluminium foil is held ~1 mm above a Kapton sheet by a gold-coated brass spacer grid in a 1-m^2 rectangular array with an effective detection area of 0.4 m^2. An actual exposure time to space, computed from the shuttle manoeuvring records provides an area–time product of 1.52×10^5 m^2 s (corrected for Earth shielding). Penetration experiments[5] on identical aluminium foils with iron microparticles show that dust particles with masses $>10^{-12}$ g will marginally penetrate the 5-μm aluminium foil. Particles of lower density would have a larger mass threshold, scaled, for example[6], according to a dependence on (projectile density)$^{1/2}$. For marginal perforations, expansion velocities behind the foil are reduced to some small fraction of the initial impact speed, but for those typical of the larger perforation, the particle totally vaporizes[7] and expands into the capture cell with little reduction of velocity. Impact debris and vapour strike the Kapton sheet over an area much greater than the area of the first perforation. Both particulate matter and vapour from the impact will adhere to the Kapton, but lighter volatiles may condense on both inner surfaces of the capture cell.

Impact perforations in the returned foils were located in the laboratory by a visual inspection system on a light table: preflight records of defects in the foils were compared with identical post-flight inspection and locations of candidates for possible hypervelocity perforations then fully examined in a scanning electron microscope. Tears and defect-associated perforations were rejected. A sensitivity of 3 μm diameter perforation is superior to the lower limit of perforation from hypervelocity impact[4], thus affording confidence that only four perforations were accumulated.

Figure 1 shows the 'entrance' side of the largest perforation and Fig. 2 the rear (exit) side of all four microparticle perforations. The exit sides show most striking morphological characteristics and also differences between the four events. The entrance morphology of all perforations is very similar, having circular lips extending away from the foil surface as seen in laboratory simulations[8].

The first and largest perforation detected (Figs 1, 2*a*) possesses very distinctive hypervelocity impact features, as would be expected from a comparatively large cosmic dust particle. Lips around the hole on both the front and rear foil surfaces are almost identical and demonstrate clear evidence of expansion after the passage of the particle, a feature shown in computer simulations[7] of impact at 68 km s^{-1}. Radial symmetry is observed and absence of deformation away from the crater lips gives evidence of a particle considerably smaller than 23 μm diameter. On the rear side of the aluminium foil, near the crater, an area of debris is seen, approximately circular with a diameter of 100 μm and centred on the perforation. It consists of submicrometre globular particles and micrometre-sized irregular iron-rich particles and may arise from the condensation of impact vapour. It could also contain 'splash-back' from the Kapton as well as condensed foil material. With this perforation, an extensive impact debris zone was found on the underlying Kapton sheet (Fig. 3), of diameter 200 μm but penetrating only to a depth of 1 μm—a marked demonstration of a meteor bumper dissipating the energy of an incident particle and of a high impact velocity. This was offset by some 500 μm from the top crater; from the concentric distribution relative to the perforation rather than the debris, we conclude that it is possible for a fraction of the expanding projectile and target to condense on the rear side of the first foil, but certainly most material impacts the Kapton first.

Returning briefly to the morphology of the three marginal perforations (Fig. 2*b*, *d*), distinctive petalling of the perforation lips and often a separation into layers is observed. This is not seen in laboratory simulations (performed with a 2-MV van der Graaff dust accelerator) at this scale. The origin of the feature seen in Fig. 2*c*, thought perhaps to be an exploded liner of a compressed micrometeoroid, is also unknown, but from energy-dispersive X-ray spectroscopy (EDS) analyses no material other

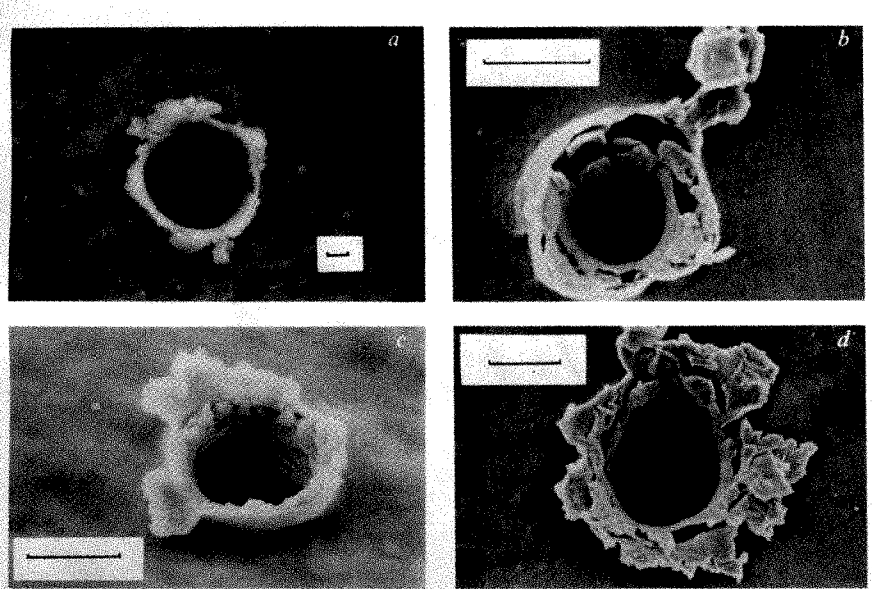


Fig. 2 Exit side of the four perforations showing: *a*, the largest perforation (23 μm); *b–d*, three near-marginal perforations of diameter 5.1, 6.5 and 7.5 μm, respectively. Although strikingly different in morphology and unmatched in microscale impact studies, *b–d* retain evidence of a melted surface of the primary crater 'liner' which is peeled open at the terminal stage of perforation. *c* could show evidence of particle retention comprising a liner of some 1 μm thickness. Scale bars, 5 μm.

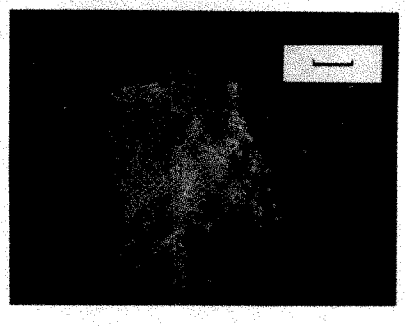


Fig. 3 Second surface debris from the largest crater collected on the Kapton substrate. Damage extends to an area of 200 μm in diameter, but is restricted to 1 μm in depth. Trails of droplets (identified as aluminium from the target) are seen forming both radial streamers and in a hollow conical distribution near the edges of the debris. Scale bar, 50 μm.

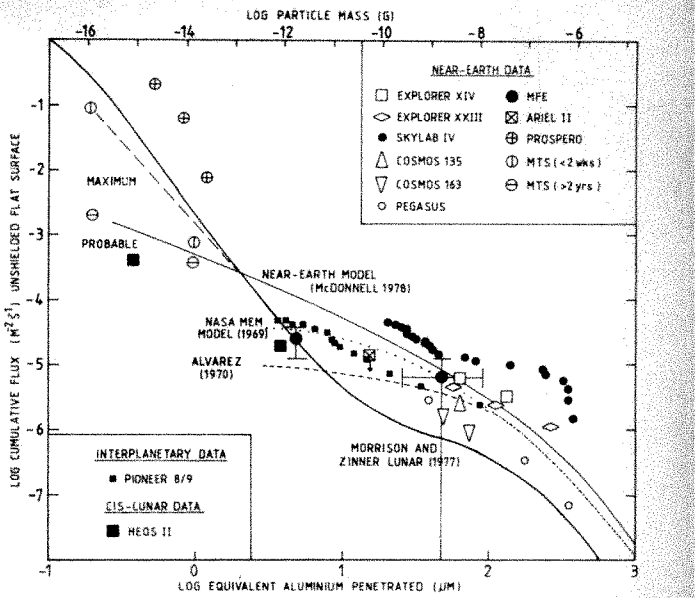


Fig. 4 Relevant flux measurements in the vicinity of 1 AU heliocentric distance[3] corrected for Earth shadowing and reduced to a flat surface exposure geometry. The MFE yields a flux of $\Phi = 2.6\times10^{-5}$ m^{-2} s^{-1} (flat surface sensor of π sr effective exposure angle corrected for Earth shielding) for a perforation thickness of 5 μm aluminium, or $m = 1.8\times10^{-12}$ g particle mass; it differs significantly from previous models and supports a more pronounced flattening of the size distribution at masses 10^{-12} g. If the lunar data and the near-Earth Prospero measurements are reliable indicators of the incident flux in Earth orbit, the trend would (shown above on a cumulative plot) support the existence of a bimodal particle distribution[12]; the significance of secondary cratering in lunar data at low masses must be addressed.

than aluminium has been detected; it may represent a mixed projectile and target with sufficiently different thermal properties to differentiate on cooling. Initial EDS examinations have, however, revealed silicon surrounding these structures. Full results of these analyses is continuing and will be reported elsewhere, as will a more detailed morphological discussion of the mechanics of penetration. Examining briefly the flux rate measured during the mission, however, we find the data offers significant insight into what could be a different understanding of some near-Earth microparticle flux data.

Figure 4 transposes the penetration data to a mass scale based on penetration experiments[5] and significant data from deep space and lunar measurements at 1 AU heliocentric distance. Two factors concerning exposure geometries must be clarified before the fluxes may be validly compared, namely sensor angles and flux isotropies. The MFE experiment offers unshielded 2π sr geometry and may be compared with data such as those of the lunar surface without geometric corrections. This configuration has generally been referred to as an area–time product in m^{2}s 2π sr, and yet considering an element of solid angle $d\Omega$ of a sensor A m^2 exposed at an angle θ of a unit sphere, we note that the product of the effective area (A_{eff}) and solid angle ($d\Omega$) is $A_{eff}d\Omega = A\cos\theta\, 2\pi\sin\theta\, d\theta$. Hence the total area–time product is

$$\int_0^{\pi/2} A_{eff}\, d\Omega = 2\pi A \int_0^{\pi/2} \cos\theta \sin\theta\, d\theta = \pi A \quad \text{m}^2\,\text{sr}$$

We should thus relate an unshielded flat sensor to a true exposure solid angle of π sr. We therefore now scale the directional measurements from sensors of solid angle Ω_p by the ratio π/Ω_p and note that the lunar data need not be changed numerically, but do refer to π sr exposure. The Pioneer 8 and 9 deep space interplanetary data were previously incorrectly scaled[3] by the ratio $2\pi/\Omega$. Such corrections have the effect of reducing measurements of the interplanetary flux by a factor of 2 (Fig. 4). For anisotropic fluxes, we note that at masses of $\sim10^{-11}$ g or greater, the data show[3] a strong predominance towards an Earth apex flux from the forward orbital motion of the near-circular orbit of the Earth into slower particles near their aphelion[9]. To such a flux, a rotating sensor of area A offers an effective area of

$$\frac{2}{\pi}\int_0^{\pi/2} A\cos\theta\, d\theta = \frac{2A}{\pi}$$

over angles $\pm\pi/2$ radians to the apex direction and 0 for $\pm\pi/2$ to $\pm\pi$ radians (shielded), yielding an average of A/πm^2. Hence, to estimate the average flux intercepted by sensor rotating in an apex dominant 'stream' we must multiply such measured apex flux values by $1/\pi$. Gravitational enhancement[10] in comparing lunar and near-Earth data must be considered, but this is not included in Fig. 4.

The relationship between foil thickness penetrated and particle mass/crater pit diameter is found[5] by assuming a particle density of 3 g cm^{-3} and a velocity of 20 km s^{-1}, which results in a 1-μm crater pit diameter being equivalent to 2.33 μm of aluminium. The MFE data in Fig. 4 are plotted at a measured cumulative penetration flux for $t = 5$ μm foil thickness for the four perforations; also estimated is the foil thickness which the particle forming the largest perforation would have perforated, namely $t = 48$ μm. This perforation is estimated by impact data[5] to have been caused by a particle of diameter 10 μm at an assumed velocity of 15 km s^{-1}. A recent near-Earth flux model[3], which interpolated through the region of sparse penetration data, is now seen to have been overestimated at masses in the region of 10^{-12} g, probably by one magnitude and at least by a factor of five. Despite the small number of hypervelocity events recorded by MFE, we stress that penetration is a highly reliable sensing technique. An exaggeration of the flux value may occur if, for example, foil defects are falsely attributed to extraterrestrial impacts, but the crater morphology precludes this possibility; scanning by two observers has established the efficacy of the MFE detection with regard to the possibility of missing perforations. Even the statistical uncertainties of the flux measure based on four events, yielding an accuracy of ±50%, are insufficient to avoid clear indications that a lower near-Earth penetration environment should be considered.

In a wider context, the less sensitive Explorer and Pegasus data compare well with lunar data[9], showing a near-Earth enhancement of the flux by less than an order of magnitude. At small masses, however, the interpretation of the MFE data in conjunction with data from Heos II[11] presents some difficulty, as the *in situ* measured flux is clearly lower than the lunar data of Morrison and Zinner[9]. Le Sergeant and Lamy[12], in considering the 'knee' of the lunar distribution, suggested that the derived primary microcrater distribution of Flavill *et al.*[13] supported the presence of two different interplanetary dust populations as first proposed by Brownlee *et al.*[14]. This work[13] decoded the obser-

ved microcrater distribution on lunar sample 12054 to remove the contribution from secondary craters, resulting in a flatter slope for the distribution in the region of 10^{-12} g. In a similar manner, an attempt has been made[15] to deconvolve the lunar data at lower masses leading to a reduction in the observed flux of a factor of 2 at a mass of 10^{-13} g and an order of magnitude at a mass of 10^{-16} g.

The need for such possible corrections to the microparticle influx derived from observed lunar microcrater distributions at masses $<10^{-12}$ g is now firmly supported by the MFE data. The experiment also exposes difficulty in understanding how the Prospero flux at mass 2×10^{-14} g is 2.5 magnitudes higher than the MFE data at mass 10^{-12} g. A logarithmic cumulative mass slope of ~1.5 is called for which is higher than any measured interplanetary or lunar microparticle flux data distributions. Although changing particle density could affect the comparison of penetration and such impact plasma experiments, the agreement between MFE and the Heos II plasma derived data argues against this, as does evidence from lunar microcraters where extremes of particle density or shape factor are shown to be atypical[16].

Impact craters recovered from Skylab windows[17] which showed evidence of aluminium microspheres in Earth orbit probably derived from upper-stage rocket firings, shows (Fig. 4) a flux distribution of anomalous slope and magnitude. The MFE data argue against this population being dominant at micrometre dimensions at least at the time of the STS-3 flight. The attribution of the four impacts to natural interplanetary sources, which is supported by chemical analyses, already yields a lower flux than previous data; we must conclude that at these dimensions the capture cell technique is likely to return a predominance of extraterrestrial material.

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Proposed structures for poorly characterized phases in C2M carbonaceous chondrite meteorites

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Recent investigations of meteorite matrices using analytical electron microscopy and high-resolution transmission electron microscopy (HRTEM) have provided data on the structure and chemistry of poorly characterized phases (PCP)[1]. We suggest here that ~10 Å PCP, a dominant matrix variety, has a structure equivalent to iron-rich tochilinite [$6Fe_{0.9}S$ 5(Fe, Mg) $(OH)_2$] which consists of coherently interstratified mackinawite and brucite sheets[2]. In addition, we propose that ~17 Å PCP, previously described as an SBB-type mixed-layer structure[3], is a commensurate intergrowth of serpentine and tochilinite layers. A wide range of cation substitutions is possible within both tochilinite and serpentine–tochilinite structural types. We suggest that various forms of PCP observed in carbonaceous chondrites[4,5] are intergrowths of tochilinite, serpentine, serpentine–tochilinite and/or valleriite-type minerals[6].

Early HRTEM studies of the Murchison C2M meteorite[7,8] revealed that the fine-grained matrix (<1,000 Å) has a complex mineralogy containing unusual mixed-layer structures, including an ordered variety termed an SBB-type structure (The term SBB-type structure indicates an ordered arrangement of serpentine-type and brucite-type layers as originally suggested by Mackinnon and Buseck[3]. Tomeoka and Buseck[9] denote this phase '7-5-5'; the numbers refer to the lattice fringe spacings in a HRTEM image.) Subsequent work confirmed the presence of SBB-type phases in the Murray, Nogoya and Yamato-74662 meteorites[10,11] and indicated that Fe may be abundant in this type of phase. Fine-scale chemical analyses (<500 Å) showed that Ni, Al, Si and S are also present[12]. Mackinnon[12] suggested that SBB-type structures in the Mighei matrix may be a variety of PCP or PCP intergrown with matrix phyllosilicates. Subsequently, detailed HRTEM studies by Tomeoka and Buseck[9,13] have shown that Mighei matrix PCP includes (1) coherent intergrowths of S-type (~7 Å) and B-type (~5 Å) layers as an SBB-type structure, (2) disordered ~5 Å and ~7 Å layers and (3) an ordered ~5 Å phase. Recent lattice image studies of C2M matrices[13,14] have emphasized an ordered ~5 Å phase in their analyses of PCP, although there is some ambiguity in the terminology used.

A compilation of data (colour, optical anisotropy, reflectivity, hardness and morphology) for typical PCPs from C2M meteorites[4,5,15] and for terrestrial tochilinite[16] shows that in all cases, properties of fibrous PCP match the bulk properties of fibrous tochilinite. A similar case can be made for the many varieties of PCP and tochilinite. Electron microprobe analyses[5] have shown that PCP morphologies are correlated with composition: a massive, sulphur-rich Fe, Ni phase; a granular to formless Mg, Si-rich Fe sulphide and a fibrous Fe, Ni phase with intermediate amounts of Si, S and C. Tochilinite is usually fine-grained in terrestrial occurrences and is difficult to analyse accurately; representative microprobe analyses from two localities are given in Table 1. For comparison, columns 3 and 4 of Table 1 also show microprobe analyses for typical PCP phases in C2M chondrites. In general, major element chemistry for tochilinite agrees well with analyses of PCP from C2M meteorite matrices.

In their study of the ~10 Å PCP phase, Barber *et al.*[1] present a set of interplanar spacings from a (presumed) ordered single crystal. These spacings, obtained from conventional electron diffraction and optical transforms of structure images, are not as precise as X-ray powder diffraction data. Nevertheless, a compilation of interplanar spacings for tochilinite[16] and the ~10 Å PCP described by Barber *et al.*[1] show remarkable agreement (Table 2). One exception in this compilation is the 7.7 Å (and perhaps 2.9 Å) spacing(s), which may be due to a small amount of serpentine-type mineral interstratified with the ordered ~10 Å PCP phase. For comparison, Table 2 also gives interplanar spacings for lizardite[17] and valleriite[18]. X-ray patterns from a fibrous variety of PCP in Murchison also give a sharp line at 5.4 Å and a weaker line at 2.72 Å (ref. 4). Barber *et al.*[1] suggest a chemical formula of $Fe_{1.4}SO_{1.3}$ for ~10 Å PCP using electron energy loss spectroscopy analysis. This method is not as precise as conventional electron microprobe analysis[1],

Table 1 Reported microprobe analyses of terrestrial tochilinites and meteoritic PCPs

	Tochilinite[16]		Meteoritic[5]	
Wt%	1	2	3	4
Fe	44.92	35.27	40.03	41.97
Mg	10.94	13.24	0.42	0.34
Al	—	4.27	0.28	0.05
S	21.73	24.33	17.00	20.40
Cr	—	—	2.26	—
Ni	—	—	7.70	5.8
C	—	—	2.2	2.4
Other	—	—	1.33	0.45
Total	77.59	77.11	71.22	71.41

'Other' includes minor amounts of Ca, K, P and Si. Columns 3 and 4 are PCP type I and PCP from a CM clast in the Jodzie meteorite, respectively[5]. Analyses from ref. 5 are converted to wt% elements to facilitate comparison with tochilinite values. All columns do not add up to 100% due to the presence of structural water which has not been analysed. For example, in column 1, a calculated (OH) content of 20.99 wt% yields a total analysis of 98.58%[16].

but within the relative error of the technique (~16%) and expected chemical variations (see below), there is agreement with the accepted tochilinite formula. Indeed, based on this analysis, Barber *et al.*[1] suggest that ~10 Å PCP may be the fibrous mineral described by Jambor[16], subsequently identified as tochilinite[2].

Selected area electron diffraction studies[2] show the complexity of valleriite- and tochilinite-like minerals, which consist of alternating sulphide and brucite sheets. Figure 1*a* shows an idealized model of the tochilinite structure along the *c* axis. In this structure, brucite-type sheets alternate with mackinawite-type sulphide sheets to form a C-centred unit cell with dimensions $a = 5.37$ Å, $b = 15.60$ Å, $c = 10.72$ Å and $\beta = 95°$ (ref. 2). The mackinawite sheet consists of FeS_4 tetrahedra in a unique edge-shared arrangement. The relative rotation of neighbouring brucites sheets is 22°, while each brucite sheet is also rotated relative to the origin of the mackinawite sheet[2].

Individual sheets within the tochilinite structure can be considered as being structural units bound together primarily by hydrogen bonds. Consequently, cation substitutions common to brucite and mackinawite can be expected for the compositional range of tochilinite. Chemical formulae on the right-hand side of Fig. 1*a* show the major cation substitutions expected within each sheet of the tochilinite structure. The mackinawite sheet can accommodate a high concentration of vacancies as well as substitutions of other cations[19]. Analyses of Kamaishi mine samples[19] show that the tochilinite structure allows significant Cu substitution within individual mackinawite sheets. However, higher contents of Cu within the sulphide sheet stabilize the valleriite structure. In valleriite, the sulphide sheet is a derivative of the cubic close-packed chalcopyrite structure containing edge-shared Cu, Fe tetrahedra[20]. Analyses of other tochilinites from Kamaishi indicate that considerable amounts of Fe and minor amounts of Al and Ca may substitute into the brucite sheet[19]. Indeed, the replacement of Fe for Mg in the brucite structure is a well-documented phenomenon and may range from 6 to 72 mol% $Fe(OH)_2$ (ref. 21 and refs therein); the latter is known as amakinite[22]. Within tochilinite, vacancies in the sulphide sheet produce a net negative charge, but this can be balanced by the presence of trivalent Fe in the brucite sheet[2,19]. Addition of minor amounts of Ni within the sulphide sheet may allow unusual charge balance distributions which may depend on the environment of formation. On the basis of carbonaceous chondrite chemical analyses[4,5], we suggest that meteoritic tochilinite will tend to form iron-rich tochilinite structures with alternating mackinawite and amakinite sheets.

HRTEM observations show that ~10 Å PCP forms sinuous, curved, rolled and 'corrugated' structures in the Mighei matrix[1,13,14]. These observations of layer curvature can be explained by the degree of misfit between two sheets of different

Table 2 Reported interplanar spacings (Å) for meteoritic PCP and terrestrial layer structure minerals

Meteoritic PCP* (ref. 1)	Tochilinite † (ref. 16)	Valleriite† (ref. 18)	Lizardite† (ref. 17)
10.8	10.84	11.4	
7.7			7.4
5.4	5.41	5.71	
	4.13		4.6
3.6	3.61	3.80	3.9
3.5	3.46	3.27	3.67
	3.15	3.23	
2.9	2.96	3.07	2.875
2.7	2.625	2.846	2.663
	2.562	2.604	2.505
2.4	2.336	2.346	2.410
	2.307		2.307
2.25	2.227	2.259	2.156
2.1	2.103		
	2.072	2.041	
	2.051	1.885	1.945
	1.861	1.860	1.835
	1.814	1.780	1.799
	1.751		1.743
1.65	1.616	1.629	1.692

* Electron diffraction data.
† X-ray diffraction data.

sizes. Within individual sheets, cation substitution will change the degree of misfit, and thus the radius of sheet curvature will also vary. The greatest degree of misfit is along the *a* axis of tochilinite[2]. Therefore, varieties of tochilinite curved about the *a* axis will be most common. In addition, the presence of edge dislocations in a plane parallel to the *a* axis of brucite provides a mechanism for the lattice to curve about *a* (ref. 23). Thus, buckling of the tochilinite structure normal to *c*, but not parallel to *a*, will produce a corrugated structure similar to antigorite[24]. However, unlike antigorite, the mechanism of misfit need not be periodic within the brucite sheet of tochilinite. Thus, corrugations within tochilinite should be limited. Buckling of tochilinite, in concert with cation substitution, may produce a three-dimensional incommensurate structure with layers loosely held

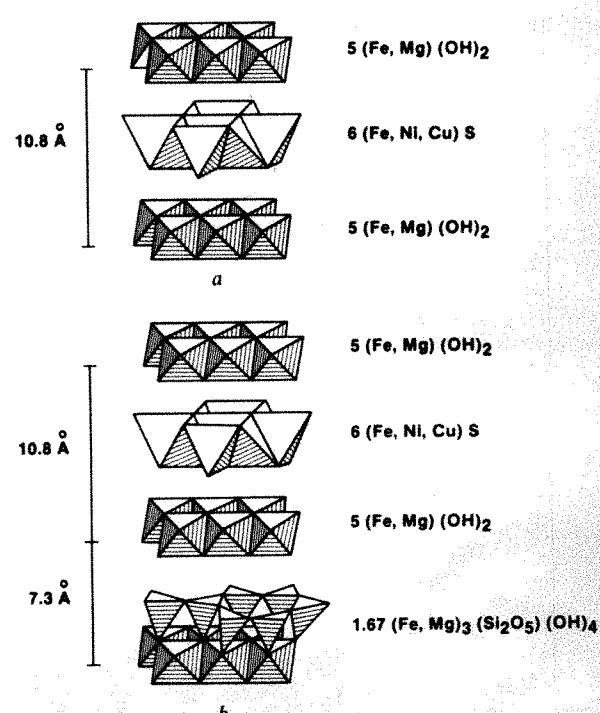


Fig. 1 Schematic models of proposed structures for two predominant types of PCP in C2M meteorites: *a*, a tochilinite structure; *b*, a serpentine–tochilinite structure.

together by hydrogen bonding. Complex structural adjustments of this nature will result in equally complex HRTEM images of meteoritic tochilinite as shown by Barber *et al.*[1] and Tomeoka and Buseck[13,14].

We further suggest that the tochilinite structure is a subunit of the SBB-type (~17 Å PCP) phase[9]. HRTEM images of ~17 Å PCP show a distinctive alternation of ~7 Å and ~5 Å lattice fringes of the type SBB (refs 4, 25) or 7-5-5 (ref. 11). These HRTEM images were initially interpreted as ordered alternations of serpentine-type (S) and brucite-type (B) layers on the basis of structural arguments and matrix bulk chemistry[3,25]. A Si-rich component of some PCPs is also inferred from their relatively high Si contents[5,12]. A combination of serpentine and tochilinite layers (held together by hydrogen bonds) gives a basal periodicity between ~17 and ~18 Å, depending on the strength of interlayer bonds and/or chemistry. A schematic model of this proposed serpentine–tochilinite structure is shown in Fig. 1*b*. In this particular case, the planar lizardite layer conforms with the observed planar layer structure of an SBB-type PCP shown in Figs 4 and 5 of ref. 25. Compositional variations within the serpentine layer may also induce a corresponding modulation of the overall structure[25]. Thus, serpentine–tochilinite structures with a higher Mg content in the 7 Å layer tend to form curved or cylindrical structures as shown in Fig. 1 of ref. 8. However, structural misfit between the mackinawite and brucite sheets of tochilinite can also produce curved, sinuous or 'turbostratic' structures. Therefore, we suggest that variations in *c* axis structure images of serpentine–tochilinite are due to the misfit of octahedral and tetrahedral sheets within both tochilinite and serpentine layers and may also correspond to subtle variations in composition along the layers.

An indication that serpentine–tochilinite may occur in terrestrial rocks comes from studies of a calcite healed breccia in limestone at the Grace Mine, Pennsylvania[16]. In these samples, some regions containing tochilinite show a distinctive compositional zoning in which optically darker bands are rich in silica and relatively low in sulphur. Jambor[16] suggests that these zones consist of intimately interlayered tochilinite and serpentine. Microprobe analyses from these regions are similar to analyses of Si-rich PCP in C2M meteorites[5]. We suggest that a detailed HRTEM investigation of Grace Mine samples will provide evidence for a terrestrial occurrence of serpentine–tochilinite. Tochilinite has also been found with a valleriite-like carbonate phase[6]. The presence of carbon in PCPs was suggested by Ramdohr[15] and concentrations as high as 8 wt % have been analysed in PCPs[5]. On the basis of these analyses and the proposed structures of two types of PCP, we suggest that valleriite-type layer structures may also occur in C2M matrices.

Conclusions regarding the petrogenesis of meteoritic tochilinites and related structure types must be developed with caution. Significant compositional differences are apparent between terrestrial and meteoritic tochilinites. For example, Ni is absent from many terrestrial occurrences of tochilinite[22], although we consider it possible that minor amounts of Ni can be substituted into the mackinawite sheet. (An indication that Ni-rich tochilinite occurs in terrestrial rocks has recently been given by van der Vusse and Powell[26].) The presence of Ni in tochilinites may prove to be an indicator of the environment of formation. In suitable conditions, substitution of other elements (for example, K, P) may also be possible in interlayer sites. Terrestrial tochilinite has been largely ignored by petrologists partly because of its small grain size and (presumed) limited occurrence. However, PCPs (and therefore, meteoritic tochilinites) constitute a significant part of an important group of meteorites, and we urge a strong commitment towards an understanding of phase relations and probable formation mechanisms for tochilinite and serpentine–tochilinite. Furthermore, experimental data obtained in conditions pertinent to carbonaceous meteorite formation should provide significant insight into the origin and evolution of C2M meteorite matrices.

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Does the photochemistry of the troposphere admit more than one steady state?

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Nitrogen oxides are released to the troposphere primarily as NO and NO_2, relatively insoluble gases which are not effectively scavenged by clouds and precipitation[1]. Nitrogen oxides are removed from the troposphere primarily as HNO_3, the highly soluble product of reactions with O_3 and various free radical species[2]. The NO_x (= NO + NO_2) removal rate thus depends on the oxidizing potential of the troposphere, which in turn is sensitive to the NO_x concentration[3]. Here we demonstrate that in certain conditions the NO_x removal rate is a non-monotonic function of NO_x concentration. A consequence is that NO_x emission rates within a critical range can support two or three different steady-state NO_x concentrations. The 'nonstandard' steady states are characterized by low concentrations of ozone and radicals, and by high accumulations of the trace gases these normally scavenge. The emission rates required to support such alternative steady states appear to be somewhat in excess of present levels.

The possibility of multiple steady states is illustrated here in a zero-dimensional (box) model, transport being neglected to emphasize the essentially chemical nature of the phenomenon. Conditions in the box are chosen to be representative of the free troposphere[4]: 273 K, 1.7×10^{19} molecules cm^{-3} (4 km altitude), and 3,000 p.p.m. water vapour.

Table 1 presents the gas-phase reaction scheme, condensed where possible by the elimination of intermediates with unambiguous fates. The rate constants are adapted from Hampson and Garvin[5] and Chameides[6]; photolysis rates represent equinoctial diurnal averages at 45° N. The effects of heterogeneous reactions are lumped into the first-order rates for physical removal, which are presented in Table 2 together with external source strengths. The degree of nonlinearity in the dependence of NO_x removal on NO_x concentration, and hence the potential for multiple steady states, is controlled by the relative importance of the (nonlinear) chemical and (linear)

Table 1 Reaction scheme used in calculations

	Reaction	Rate coefficient
R1	$NO+O_3 \rightarrow NO_2$	1.5 (+1)
R2	$NO_2+h\nu \rightarrow NO+O_3$	1.6 (−1)
R3	$NO_2+O_3 \rightarrow NO_3$	2.2 (−2)
R4	$NO_3+NO \rightarrow 2NO_2$	2.8 (+4)
R5	$NO_3+h\nu \rightarrow NO_2+O_3$	1.4 (−1)
R6	$NO_3+h\nu \rightarrow NO$	1.4 (−1)
R7	$O_3+h\nu \rightarrow 2HO$	9.4 (−6)
R8	$CO+HO \rightarrow HO_2$	4.5 (+2)
R9	$CH_4+HO \rightarrow CH_3O_2$	6.6
R10	$C_2H_4+HO \rightarrow 2CH_2O+HO_2$	1.3 (+4)
R11	$CH_2O+HO \rightarrow CO+HO_2$	1.8 (+4)
R12	$CH_2O+h\nu \rightarrow CO+2HO_2$	4.3 (−4)
R13	$HO_2+NO \rightarrow NO_2+HO$	1.2 (+4)
R14	$CH_3O_2+NO \rightarrow CH_2O+HO_2+NO_2$	7.8 (+2)
R15	$HO+NO_2 \rightarrow HNO_3$	1.8 (+4)
R16	$HNO_3+h\nu \rightarrow HO+NO_2$	6.6 (−6)
R17	$HO+O_3 \rightarrow HO_2$	5.7 (+1)
R18	$HO_2+O_3 \rightarrow HO$	1.0
R19	$2HO_2 \rightarrow H_2O_2$	3.7 (+3)
R20	$H_2O_2+HO \rightarrow HO_2$	9.5 (+2)
R21	$H_2O_2+h\nu \rightarrow 2HO$	3.7 (−5)
R22	$HO_2+HO \rightarrow$ —	4.4 (+4)

Rate coefficients are adapted from Hampson and Garvin[5] and Chameides[6] for the following conditions: density = 1.7×10^{19} molecules cm^{-3} (z = 4 km), T = 273 K, water vapour = 3,000 p.p.m., equinox at 45° N. Numbers in parentheses are powers of 10; units are min^{-1} or p.p.m.$^{-1}$ min^{-1}.

physical removal pathways. Accordingly, two sets of physical removal rates are considered, one representing relatively long (500 days for NO), the other relatively short (10 days for NO), lifetimes against physical removal. The source strengths of O_3, CO, C_2H_4 and CH_2O in Table 2 are obtained by distributing the estimated global emissions uniformly (with respect to mixing ratio) throughout the troposphere. The source strength of NO_x is varied as a parameter, the composition of the emissions being held constant at 95% NO and 5% NO_2. Only the methane concentration is externally imposed, at 1.6 p.p.m.; all other concentrations are determined by the equilibration of sources and sinks.

Figure 1 shows how the calculated steady state varies with NO_x concentration for a weak physical sink. Photolysis of O_3(R7) yields HO, which reacts with CO and hydrocarbons (R8, R9, R10) to yield HO_2 and CH_3O_2, which oxidize NO (R13, R14) to NO_2, which photolyses (R2) to yield O_3. In the natural troposphere NO_x is the limiting reactant for the photochemical production of O_3 (ref. 3), and the oxidizing potential of the troposphere increases with increasing NO_x concentration. Because the photostationary ratio of NO_2 to NO increases with O_3 (ref. 7), NO_2 must increase with NO_x as long as O_3 does. As the ratio of NO_2 to CO and hydrocarbons increases, however, so does interdiction of the photochemical chain by R15, a non-productive sink for HO. The NO_2 concentration cannot continue to rise indefinitely without reducing the O_3 yield on O_3 photolysis below break-even. At some point, therefore, O_3 must begin to decrease with further increases in NO_x. Figure 1 shows that a break in fact occurs in the neighbourhood of 1 part in 10^9 (p.p.b.) NO_x. Increases in the NO_x concentration beyond this level produce a decrease in the ozone concentration and an even stronger decrease in free radical concentrations, as the growth of sinks (R13, R15) compounds the decline of the source (R7). Similar calculations by Stewart *et al.*[8] and Hameed *et al.*[4] have yielded the same general pattern, with O_3 and HO peaking at slightly lower NO_x concentrations. An analogous nonlinearity in the chlorine chemistry of the stratosphere has been demonstrated by Prather *et al.*[9] and Fox *et al.*[10].

Figure 2 shows the effect of the break in photochemical reactivity on the NO_x removal rate. As a function of NO_x

Table 2 External source and sink strengths used in calculations

	Source Q (p.p.b. min^{-1})	Sink $1/R$ (days)	
		Weak	Strong
NO	Variable	500	10
NO_2	Variable	50	10
O_3	3×10^{-4}	50	10
CO	7×10^{-4}	500	500
C_2H_4	5×10^{-6}	50	10
CH_2O	6×10^{-7}	50	10
CH_4	Concentration imposed		
HO	0	10	1
HO_2	0	10	1
CH_3O_2	0	10	1
H_2O_2	0	10	1
NO_3	0	10	1
HNO_3	0	10	1

Source strengths of O_3, CO, C_2H_4 and CH_2O represent estimated global emissions[6,19,20], distributed uniformly (with respect to mixing ratio) throughout the troposphere. The source strength of NO_x is varied as a parameter, with the composition of the emissions held constant at 95% NO and 5% NO_2. The methane concentration is fixed at 1.6 p.p.m. Sink strengths, R, are the first-order coefficients for removal by rainout and other mechanisms not included in the reaction scheme. Two sets of sink strengths are used, presented here in terms of lifetimes, their reciprocals.

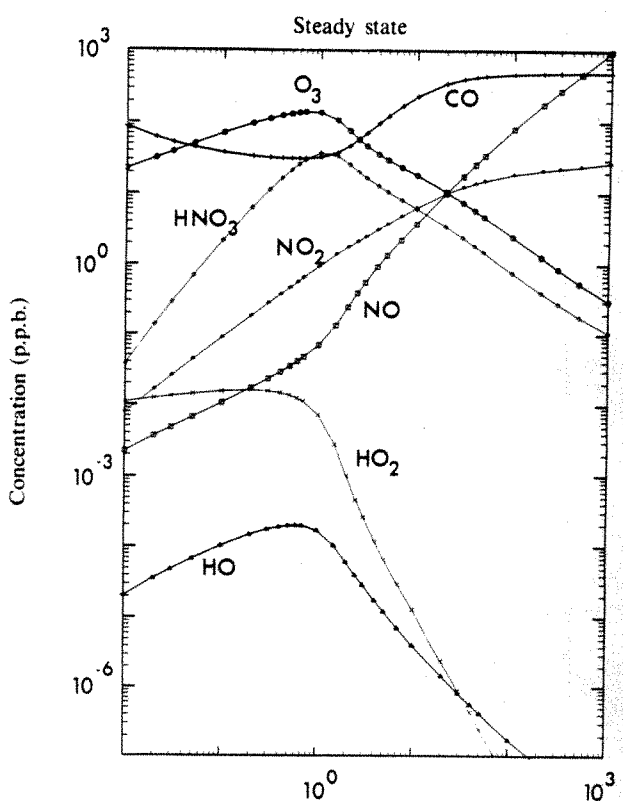


Fig. 1 Calculated steady state of the free troposphere as a function of NO_x ($=NO+NO_2$) concentration. Conditions are those of Tables 1 and 2, with a weak physical sink.

concentration, the NO_x sink has a relative maximum where the reactivity peaks, and a relative minimum where oxidation and physical removal are of comparable importance. A similar pattern is observed for a strong physical sink, with the photochemical peak slightly attenuated and the transition to predominantly physical removal occurring at lower NO_x concentration. The effect of holding the CO concentration fixed at 150 p.p.b., shown in Fig. 2 for a weak sink, is to accentuate

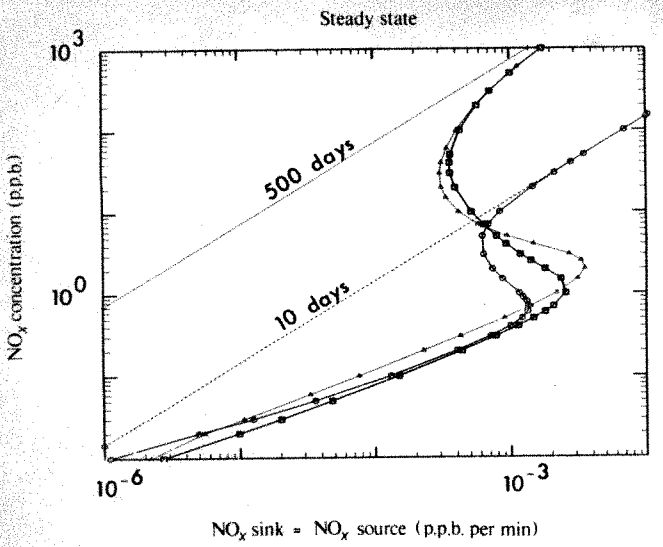


Fig. 2 Calculated steady-state NO_x ($=NO+NO_2$) concentration in the free troposphere, as a function of NO_x source strength. The heavy curves are calculated from the reaction scheme of Table 1 and the source strengths of Table 2; the one marked with circles corresponds to the strong physical sink of Table 2, the one marked with squares to the weak physical sink. The light curve (marked with triangles) shows the effect of fixing the CO concentration at 150 p.p.b., for the weak physical sink. The sloping lines, labelled by the lifetime of NO against physical removal, show the linear relationship of concentration to emissions in the absence of photochemical oxidation.

both peak and trough in the sink strength. (The free-floating calculations underestimate the CO concentration, presumably due to gaps in the inventory of emissions (compare refs 6 and 11). The non-monotonic dependence of the NO_x sink on NO_x concentration is, in sum, a fairly robust feature of the calculation. The principal limitation of this result arises from its basis in diurnally averaged photolysis rates, which do not allow for the buildup of NO_3 in the dark. The rapid equilibration of NO_3 with soluble N_2O_5 provides, in the boundary layer and in clouds, a potentially significant night-time pathway for the oxidation and removal of NO_x (refs 2, 12). The calculations presented here are therefore most relevant away from clouds in the free troposphere where, in the absence of liquid water, NO_3 and N_2O_5 simply accumulate overnight and revert to NO_x at daybreak.

It is clear from Fig. 2 that dissimilar NO_x concentrations can yield similar NO_x removal rates. As the steady-state NO_x concentration is determined by equilibration of source and sink strengths, this means that a given rate of emission may support more than one steady-state concentration. Consider a source strength for NO_x of 1 part in 10^{12} (p.p.t.) per min, for example, in combination with a strong or weak physical sink. Figure 2 shows that three distinct steady states are possible in either situation. The low-NO_x state is stable, and is characterized by concentrations which are not uncommon over industrial regions: 0.40 p.p.b. NO_x and 64 p.p.b. O_3 for a strong sink, 0.36 p.p.b. NO_x and 120 p.p.b. O_3 for a weak sink. The middle-NO_x state is unstable, and is therefore of no practical interest. The high-NO_x state is characterized by concentrations not presently seen in the free troposphere: 13 p.p.b. NO_x and 4 p.p.b. O_3 for a strong sink, 500 p.p.b. NO_x and 0.5 p.p.b. O_3 for a weak sink. The high-NO_x state is stable, however, and would be expected to persist once established in a sufficiently large region. A shift from the low-NO_x state to the high-NO_x state would profoundly change the reactivity of the troposphere, permitting the accumulation of CO and other trace gases presently scavenged by O_3 and HO (ref. 13). (One of the gases to accumulate would be CH_4, assumed in our calculation to remain at constant concentration—because of this and other neglected long-term feedbacks, the high-NO_x solution is best viewed as a quasi-steady state whose ultimate fate is difficult to predict.) An emissions transient capable of shifting a large region into the high-NO_x state is not outside the realm of possibility; Crutzen and Birks[14] calculate that nuclear war could inject of the order of 100 p.p.b. NO_x into the upper troposphere between 40° and 60° N.

In the conditions considered here, multiple steady states become possible at NO_x source strengths above 0.3–0.6 p.p.t. min^{-1}, where the minimum NO_x concentration is $\geq$0.2 p.p.b. Actual NO_x concentrations reach the 0.2 p.p.b. threshold over industrial continents[2], but tend to be substantially lower over remote areas[15]. Actual NO_x emissions are currently estimated[2] to average about 30×10^9 molecules $cm^{-2}\,s^{-1}$ at 45 °N. Much of this originates within the boundary layer, so that the effective source to the free troposphere depends on the rapidity and nature of exchange between the two layers[16]. Even if they were distributed uniformly in the vertical, however, such emissions would correspond to a source strength of only 0.1 p.p.t. min^{-1} supporting a unique steady-state NO_x concentration of ~0.1 p.p.b. It appears likely, therefore, that current emissions do not quite suffice to support nonstandard steady states on the zonal scale considered here. This is not a firm conclusion, because the viability of alternative steady states is sensitive[10,17] to details of atmospheric mixing which are poorly understood. It should also be noted that natural emissions of NO_x are rather uncertain[2], and that anthropogenic emissions are rapidly increasing[18].

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Synthesis of marine humic substances from unsaturated lipids

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The origin and structure of the dissolved blue-fluorescent yellow organic substances (Gelbstoff) in seawater have long eluded characterization[1]. The fact that this class of organic matter, which comprises 10–50% of the organic carbon in the sea[2], became known as marine humus (fulvic and humic acids) has led to much confusion because of the implied relationship with soil humus[3]. Based on chemical and spectral studies of oceanic and coastal humus we recently proposed a class structure and a mechanism

by which these marine humic substances could form[2]. The hypothesis was that they are water soluble, aliphatic organic acids formed by the autoxidative cross-linking of two or more unsaturated lipids released into seawater. We have now confirmed that hypothesis by allowing pure marine lipids and a diatom to autoxidize in seawater in the laboratory. We report here that the yellow substances obtained are similar in all respects to marine humus.

The syntheses were conducted in coastal Miami seawater from which the humus was removed by extraction with Amberlite XAD-2 at *p*H 2 (refs 2, 4, 5): 10 litres of this water were adjusted to *p*H 8.2 in an open steel container. About 50–100 mg of the particular lipid, dissolved in 5 ml of ethyl ether, were added in 5 mg aliquots to the seawater every 0.5 h until the final concentration was 5 mg l^{-1}. Stirring was continuous throughout the 24–100 h duration of the experiment with the water fully exposed to air and laboratory light. To terminate the synthesis the water was acidified to *p*H 2 and after 1 h passed through a 75 cm^3 column of XAD-2 at 60 ml min^{-1}. Isolation of the products from the resin with methanolic ammonia was by the same process as described elsewhere[2,4,5]. The pure lipids used were trilinolein, triolein, glyceryl-1,3-dioleate and glyceryl-1-palmitate-2,3-dioleate. The yields were generally greater than 50 mg. Another synthetic, fulvic acid, was made by allowing freeze-dried *Skeletonema costatum*, a marine diatom, to stir in extracted seawater for 1 week. Fully saturated tripalmitin and tristearin produced no product with the properties of marine humus.

The products obtained from the pure unsaturated lipids and the lipids in the diatom[6] were pale-yellow powders freely soluble in water but insoluble in ether. An elemental analysis of the product from trilinolein was: C, 45.73%; H, 6.71%; N, 0.86%. These values compare well with those of a fulvic acid from the Sargasso Sea[7]. The $\delta^{13}C$ of this material was −19.7‰, which compares well with the isotope values of three marine fulvic acids we isolated from the Gulf of Mexico, that is, −20.6, −21.5 and −22.0‰. Interestingly, the $\delta^{13}C$ of the trilinolein used was −28.1 but we do not know the cause of this large fractionation. Small amounts (3–5 mg) of synthetic humic acids precipitated at *p*H 2 from the trilinolein reaction mixtures.

In all cases, the proton NMR spectra of the synthetic humic substances could be matched with one or more spectra obtained from our collection of oceanic humus[2] (Fig. 1). The extent of cross-linking and heteroatom incorporation can be estimated from the relative widths of the methylene (1–2.5 p.p.m.) and H–C–O– (3–4 p.p.m.) peaks in the NMR. The IR spectrum of the product of trilinolein autoxidation is superimposable on the spectra of marine fulvics. In addition to the yellow colour of both the natural and synthetic substances, irradiation at 350 nm produces the same broad fluorescence emission between 350 and 650 nm, indicating that the same chromophores are present. Gel permeation chromatography at *p*H 4, 6 and 8 using both acetate and phosphate buffer eluents (Varian TSK 2000 SW and Sephadex G-25 columns) gave retention times of synthetic and natural fulvics within 5% of each other, indicating similar size and functionality. Although not strictly comparable, polyethylene glycol molecular weight standards indicated a molecular weight range mainly between 600 and 800, which is consistent with our proposed structures. These molecular weight results should be treated with caution because the standards were not the exact same class of compounds[8].

We have also observed the formation of synthetic marine humic substances by quantifying the development of the zinc complexing ability of seawater containing an unsaturated lipid. We have shown that marine humus is a good complexer of copper and zinc as detected by differential pulse anodic stripping voltammetry (DPASV)[9,10]. Trilinolein (2.5 mg l^{-1}) was added to aged Gulf Stream water which had little or no complexing capacity for Zn. The Zn signal was monitored periodically for 38 h by standard additions followed by DPASV analysis. The complexing capacity increased from 0.0 at 4 h to 0.5 nmol kg^{-1} at 8 h to a total of 3 nmol kg^{-1} at 38 h. The more saturated glyceryl 1,3-dioleate-2-palmitate required 48 h before any increase in Zn complexing capacity was observed. The fully saturated tripalmitin does not autoxidize and did not increase the Zn complexing capacity of the seawater. The different times required by the two lipids to autoxidize and develop Zn complexing ability are related to the number of free radical reactive sites. The synthetic fulvic acid from trilinolein was also shown to protect photosynthetic plankton from copper inhibition to the same degree as a natural marine fulvic[11].

The above evidence supports our proposed mechanism of formation and structure of oceanic humus. Humic substances isolated from estuarine and coastal waters are generally admixtures from marine, freshwater and dissolved soil sources and can have different properties from those described for ocean water. The word 'structure' in this context does not refer to a single compound but a closely related family of compounds whose properties can be described by one or two structural formulas as is the case with coal and lignin.

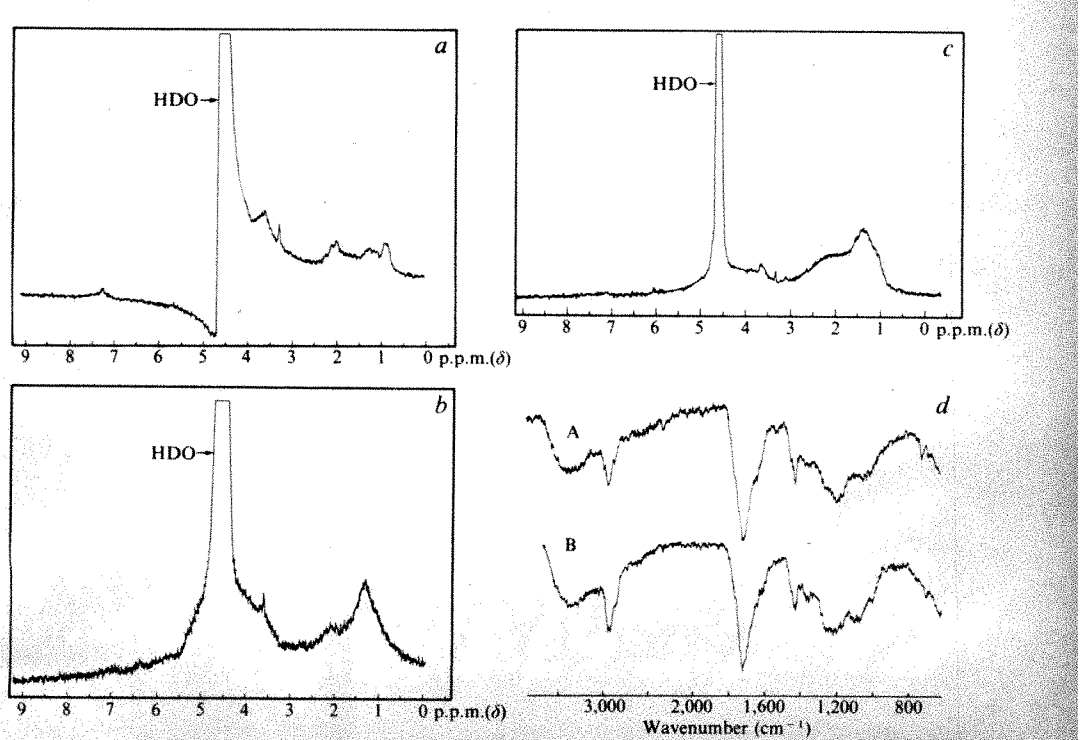


Fig. 1 Proton NMR spectra of the synthetic fulvic acids from trilinolein (*a*), glyceryl-1,3-dioleate-2-palmitate (*b*), and an open ocean fulvic acid (*c*). The IR spectra of the methyl esters of the marine fulvic acid (A) and the trilinolein product (B) are shown in (*d*). Note the greater amount of unaltered methylene protons (1.2 p.p.m.) in *b* in comparison with *a*.

The reactions described here are extracellular examples of the enzyme-controlled cyclization of arachidonic acid (20:4) to prostaglandins[1,2] and the random free radical autoxidation of cellular unsaturated lipids, a process implicated in many pathologies[13].

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Ciliated protozoa associated with oceanic sinking detritus

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In the past decade, protozoa have been recognized as ubiquitous residents of the upper waters of the sea, where they have key roles in detrital and producer food webs[1–4]. In contrast, protozoa are almost unknown from aphotic depths, where food chains presumably are supported by detritus. We show here that ciliate protozoans do accompany sinking detritus to bathypelagic depths. In the productive northeastern Pacific Ocean, the biomass of ciliates on particles settling between the surface and 2,000 m far exceeds the biomass of bacteria, which are the only widely recognized detrital microorganisms in deep pelagic communities. Although absolute numbers of ciliates decline below the euphotic zone, their concentrations (number per g detritus) drop only slightly by 2,000 m. Taxonomic data and patterns of abundance[5] indicate that the ciliates are endemics and not remnants of surface colonists, and that the deep sea is a favourable environment for them. Their high concentrations, possibly together with other smaller protozoa, indicate that protists may control bacterial mineralization rates on sinking particles, thereby regulating the vertical distribution of many biologically active elements in the sea.

We collected sinking particles in drifting, preservative-filled sediment traps[6] deployed in the high productivity, eastern boundary current off central California (vertical transport and exchange of materials in the upper waters of the ocean, VERTEX I) and Mexico (VERTEX II and III). Bacteria and ciliates in the traps were counted using methods outlined in Fig. 1. The microorganisms we found were probably associated with the trapped particles, as the sinking rates of individual cells would be insufficient to account for their numbers in the collections.

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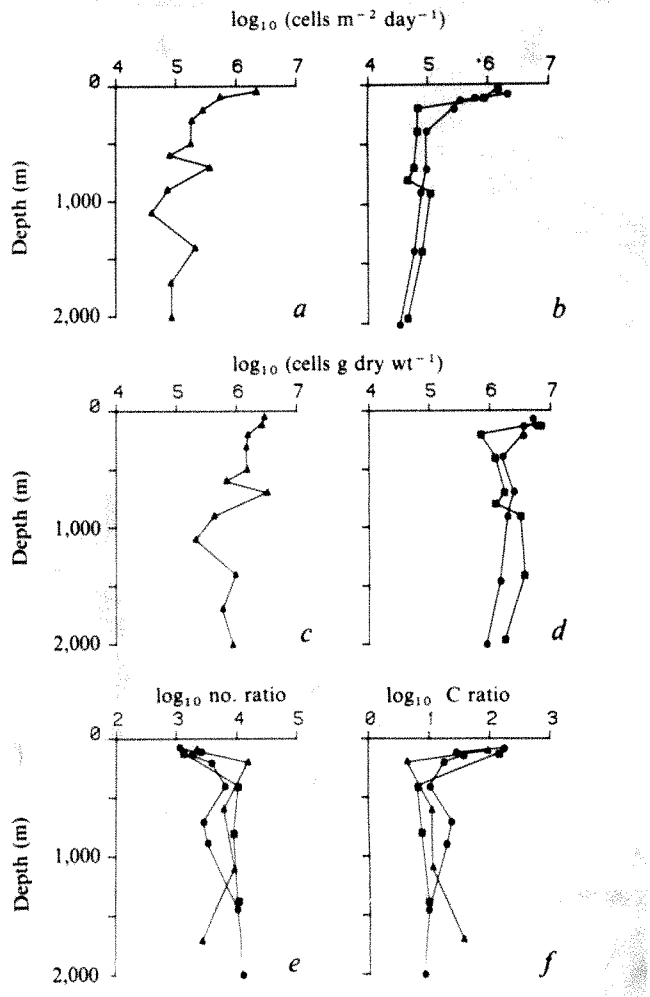


Fig. 1 Microorganisms (numbers and biomass) from sediment traps in the northeastern Pacific Ocean during VERTEX cruises. During VERTEX I (▲), traps were deployed from 26 August 1980 to 8 September 1980 at 35° N; 123° W, during VERTEX II (■) from 27 October 1981 to 17 November 1981 at 17° N; 109° W, and during VERTEX III (●) from 9 November 1982 to 30 November 1982 at 16° N; 107° W. Because they collect for several weeks, traps measure the time-averaged standing crop on sinking particles, or 'flux' of organisms: numbers m^{-2} day^{-1}. Ciliates in the traps were counted and sizes measured at ×400 in 3–5 aliquots in settling chambers with an inverted phase microscope. Specimen condition was checked in aliquots prepared for transmission electron microscopy (TEM). Protargol silver stained[13] specimens were identified using oil-immersion, bright-field optics. Bacteria stained with diamidinophenylindole[14,15] were counted on 0.2 μm Nuclepore filters at ×1,188 with oil immersion optics from three aliquots of trap material and sizes measured in TEM preparations[16]. Carbon content was estimated using carbon-volume relationships from the literature[17,18]. *a*, Ciliate flux during VERTEX I. *b*, Ciliate flux during VERTEX II and III. *c*, Ciliate flux per g dry weight of trap material from VERTEX I. *d*, Ciliate flux per g dry weight of trap material from VERTEX II and III. *e*, Ratio of numbers of bacteria to numbers of ciliates in the trap material from the three cruises. *f*, Ratio of biomass (cell carbon: see above) of ciliates to biomass of bacteria in the trap material from the three cruises.

Ciliates were most common in the shallowest traps, but flux remained above 10^4 cells m^{-2} day^{-1} at all depths (Fig. 1*a*, *b*). The decrease in trap-collected ciliates with depth largely resulted from reduction in the quantity of sinking particulate matter, since numbers per unit dry weight declined only slightly with depth (Fig. 1*c*, *d*). Ciliate flux showed no consistent reduction in the pronounced oxygen minimum between 600 and 950 m on VERTEX I (ref. 7) and between 100 and 1,000 m on VERTEX II (ref. 8) and III (W. Broenkow, personal communication). Although bacteria outnumbered ciliates by 10^3–10^4 (Fig. 1*e*),

Table 1 Ciliates* associated with vertically sinking particles† collected during VERTEX II‡ in the Pacific off central Mexico

Depth	30 m	120 m	1,950 m
Numerical dominants	(1) Oligotrichs (such as *Strombidium, Tontonia*) (2) Haptorids (such as didiniids)	(1) Scutico-ciliates (2) Protosto-matids (such as *Metacystis*)	(1) Protosto-matids (such as *Metacystis*) (2) Scutico-ciliates (3) Nassulids (4) Oligotrichs (such as *Strombidium, Strobilidium*)
No. of species per 50 specimens	19	10	15
No. of species shared between adjacent depths	0		5

At our sampling site, temperature at 30 m was 38.5 °C, at 120 m was 14.0 °C, and at 1,950 m was 2.3 °C and water depth was ~3,500 m. Major ciliate orders are listed and species diversity is indicated. Specimens were classified using the taxonomic scheme of Corliss[9].

* Some fixation-related shrinkage occurred and loss of especially delicate cells was possible; controls for possible cellular lysis in sediment traps are not available. Predeployment checks of net-collected, surface populations with a variety of fixatives, however, indicated our trap preservation solution caused comparatively little ultrastructural (TEM) damage to cells. (Species lost due to fixation could affect the apparent 'endemism' to an unknown extent. Quantitatively, however, losses would result in the underestimation of ciliate importance in our samples.)

† We assume these ciliates represent populations accompanying vertically sinking particles—that significant amounts of allochthonous particulates were not introduced at mid-depth by horizontal advection. A common surface source for the three traps was indicated by their similar phytoplankton debris. Furthermore, current flow measurements did not suggest a physical basis for the largest vertical species change—that found between populations in the two upper traps: the measured maximum current sheer[8] and the predicted sinking speed of marine snow[19] indicate a horizontal displacement of no more than 30 km between sources for the two traps. Most likely, species changes in the living microbial populations resulted from environmental stresses, particularly the low oxygen levels at mid-depth. Moreover, chemical analyses do not indicate significant new inputs of particulate materials at the two deeper trap depths[20].

‡ Although we did not perform such a detailed species analysis on materials from VERTEX I or III, a preliminary study of ciliates from the Central California Site (I) showed similar depth-related changes as were observed at the Mexican site (II) and similar ciliate species arrays at depth.

ciliate biomass exceeded that of bacteria due to the relatively large size of the protozoa (usually 10–150 μm compared with < 1 μm, respectively). The ciliate dominance was most pronounced near the surface, but ciliate biomass still exceeded that of bacteria in the deepest traps (Fig. 1*f*).

Taxonomic composition of ciliate populations changed markedly with depth (Table 1). Species richness was highest in the near-surface trap, lowest in the oxygen minimum zone, and intermediate at the greatest depth. The ciliate taxa in the shallowest trap were similar to those from the euphotic zone, but shared no species with the deeper populations. The two deep-water ciliate samples contained taxa not normally reported in lists of planktonic organisms, possessed some of the same species, and included forms that represent not only new species and genera but even higher ciliate taxa (ref. 5 and D.C.B. and J.O.C., manuscript in preparation; also see the classification used in ref. 9).

Our VERTEX results point to several conclusions. First, protozoa on sinking pelagic detritus from the deep sea are as highly concentrated per unit dry weight of detritus as those from any shallow water or laboratory detritus[10–12]. Furthermore, ciliate concentrations on sinking particles remain high with depth, demonstrating that the decline in ciliate flux is due mostly to the decrease in the quantity of sinking material. The numbers of ciliates, their ultrastructural integrity in electron microscope sections, and their possession of food materials in vacuoles suggest participation in food webs to 2,000 m. The change in species composition with depth further supports the view that the ciliates are not passive members of the rich microbial communities but are populations actively responding to environmental alterations.

The numbers of ciliates relative to bacteria (Fig. 1*e*) indicate that decomposer communities of the aphotic zone have a population composition resembling that of other detrital communities and thus may be biologically regulated by mechanisms common to the others. In well-established detrital systems, substrate decomposition is largely due to bacteria but protozoa control the rates of mineralization by the bacteria by grazing on them. A variety of protozoa are usually present in detrital communities, but the largest of these—the ciliates—play pivotal roles in structuring the communities: they affect overall decomposition rates by consuming the abundant smaller protozoa that eat bacteria as well as by directly grazing the bacteria[10–12]. The deep-water ciliates we describe seem to belong to the decomposer assemblage on particulates because they contain bacteria, detritus, and other local food materials in their vacuoles, and no other significant ciliate food resources are available at these depths: the concentrations of cells in the water (as noted from water bottle samples) are several orders of magnitude less than on the sinking particles (unpublished results from this study and ref. 19). The abundance relationships between ciliates and bacteria further argue for the regulatory role of the former: communities with numerical ratios of bacteria/ciliates ranging from 10^2 to 10^4 represent detrital systems in which bacterial production is ultimately regulated and mineralization rates stimulated by the ciliates[10–12]. Since bacterial/ciliate ratios on sinking detritus from both euphotic and aphotic zones fall within this range, an equivalent functional role for the ciliated protozoa seems likely. Such a role does not negate the importance of other, smaller protozoans including microflagellates (which were also present but not counted in the present study), which may be the primary consumers of bacteria, but ciliates are probably the group that ultimately moderates the population sizes and activities of all the lower trophic level members of the decomposer community.

Because ciliates are so much larger than bacteria, the food base that supports the decomposer community, they are available to some consumers unable to capture bacteria. Ciliates may be significant trophic intermediaries between the prokaryotes, which are well known components of deep ocean food webs, and the metazoa. Our studies suggest that the ciliates could have that role on the particulates that rain into the deep sea.

Biological materials on sinking detritus are usually thought to originate from surface waters. However, the ciliates suggest more complex interactions among living components as the particles sink. The marked changes in species composition with depth indicate either a multiple-depth origin of the detritus, a colonization of the detritus at several depths by regional endemics, or a succession in which initially rare species progressively replace the early dominants. The high diversity of the protozoa, furthermore, indicates that the detritus represents a favourable growth environment and that ciliates occupy various niches in these densely populated micro-habitats. Thus the taxonomic data, as well as the biomass measurements, show that sinking particles support active, concentrated, and biologically regulated microbial communities passing through one of the most sparsely populated environments in the biosphere.

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Remote telemetry of daily vertical and horizontal movement of *Nautilus* in Palau

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Vertical depth migrations, by the chambered cephalopod *Nautilus* when moving into shallower waters at night, were first documented by Wiley[1]. Although unsupported by quantitative data, this observation strongly influenced the interpretation of the mode of life of *Nautilus* and of the palaeoecology of fossil chambered cephalopods[2–4]. To determine whether these migrations do occur, we mounted ultrasonic transmitters on four *Nautilus belauensis* in Palau. The transmitters contained a strain-gauge depth sensor that caused the transmitters to emit sonic pulses at intervals that varied with depth, so that geographical position and depth could be ascertained. Our results indicate that at least in this population or geographical area, vertical depth changes of up to 200 m per day are common. Although too soon to make generalizations about other populations of *Nautilus* in the Pacific, it has been suggested that the morphological position of the siphuncle within the phragmocone of the chambered shell would increase the efficiency of vertical migrations in *Nautilus*, if such migrations were found to occur[5,6]. As similar siphuncle configurations are common in most post-Triassic nautiloids, and some ammonoids[6,7], it could be that vertical migration was common in many fossil forms as well.

The sonic transmitters were first calibrated in pressure tanks, and by submergence in the sea to known depths. The devices were designed to emit at different frequencies (between 38 and 41 kHz), allowing two *Nautilus* to be tracked simultaneously, and were filled with non-compressible glass microballoons to make the transmitters neutrally buoyant in seawater. The batteries gave the transmitters a life of 7–14 days, and a horizontal range of ~2 km. The transmitters sat in moulded rubber saddles that allowed them to be mounted on the tops of the shells of large, mature *Nautilus* (two males and two females). The *Nautilus* were captured at 200 m in baited traps, and after being fitted with the transmitters, immediately returned to the sea and released by divers at a depth of 70 m. All four *Nautilus* quickly descended to about 200 m, at which point the periods of observation were started. The *Nautilus* were tracked using Dukane hydrophones.

The four *Nautilus* monitored (termed Yellow, Green, Blue and Red, based on colour coding of the transmitters) were tracked for periods of between 2 and 10 days (Figs 1, 2) during June–July 1983. Completeness of tracking depended entirely on weather conditions. Conditions were nearly ideal during the tracking of Yellow, allowing continuous daytime and nighttime

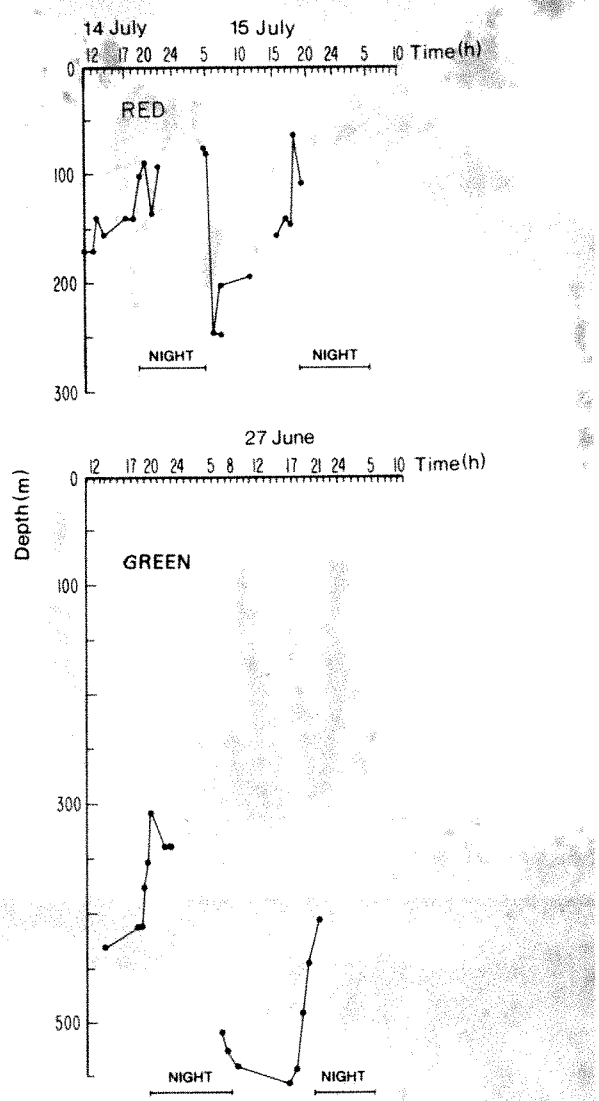


Fig. 1 Vertical movement of two *Nautilus*: Red, followed during 14–15 July 1983, and Green, followed during 27–28 June 1983.

work, but much less favourable for Green, Blue and Red, which were tracked mainly during daytime hours. All four animals showed significantly deeper mean daytime than nighttime depths (Table 1). Yellow, Red and Green showed daily ascents, occurring between 1600 and 2000, and descents between 0400 and 0700. (During our observation period (June–July 1983) sunset occurred at 1815 and sunrise at 0545.) Yellow was extremely regular in its diurnal movements. Blue was more irregular, and on three occasions (8, 10 and 13 October) showed evening descent rather than ascent. Because tracking was incomplete we do not know whether this animal ascended later on these evenings.

The tracking data indicate no daily rest periods. Although the largest scale depth displacements occurred at dawn and dusk, other vertical movements also occurred during day and night-time hours. We never observed any *Nautilus* spending longer than a few hours at the same depth; our data suggest nearly constant activity.

All four *Nautilus* showed substantial horizontal as well as vertical movement. One (Yellow) showed a somewhat regular pattern of movement around the south-west corner of Augulpelu Reef; each daylight period it moved into deeper water on the south side of the reef, while at night it moved around the point, into the large bay defined by Augulpelu and Ngadarak reefs, a distance of ~1 km. The other three *Nautilus* showed a different pattern. After being released near the point of Augulpelu reef, Green, Blue and Red began a daily southwestern movement along the 200 m contour of the bay. Superimposed

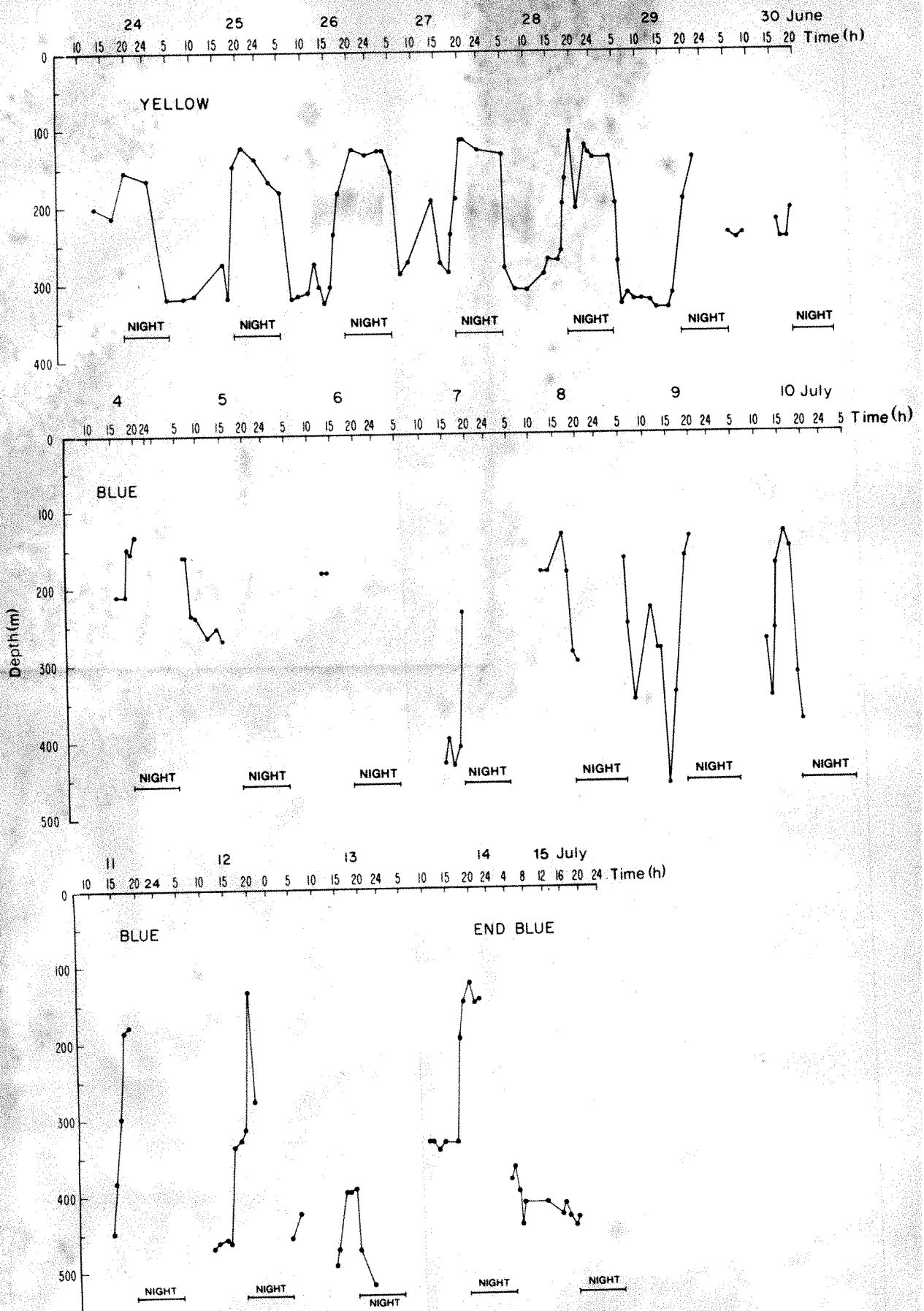


Fig. 2 Vertical movement with time of two *Nautilus belauensis*, Yellow and Blue. The ordinate records the depth, as derived from t strain-gauge depth sensor of the transmitter. Yellow was tracked from 24 to 30 June 1983. Blue was tracked between 4 and 14 July 198 Each dot represents the mean of 6–10 depth readings taken over a 2–3 min period. Numbers on the horizontal axes refer to the hour of da Periods of darkness (night) are indicated by bars at the bottom of each graph.

Table 1 Results of depth tracking for four specimens of *Nautilus*

Name	Sex	Dates of observation	Time*	Mean day depth† (m)	Mean night depth† (m)
Yellow	M	24–30 June 1983	152	290	153
Blue	F	4–15 July 1983	266	329	260
Green	M	26–27 June 1983	34	441	321
Red	F	7–15 July 1983	32	120	87

* Hours under observation.

† Derived from daytime and nighttime depth figures, with a maximum of two readings during any single hour being included. In the case of Yellow the mean depth figures were computed by integrating the depth curves.

on this southwestern horizontal movement was vertical movement, which was accomplished by inshore and offshore motion. Because of the steepness of the Palauan reefs, very short horizontal distances resulted in large vertical displacements. The Blue animal, followed for the longest period, travelled a total of 16 km along the reef front during the 10-day observation period. Horizontal movements of a similar magnitude have been observed previously for longer time periods in this population of *Nautilus*[8].

The depths at which *Nautilus* were found, as computed from the ultrasonic emissions, were compared with the bottom depth recorded nine times using a fathometer. The two depths differed significantly in only two cases, both involving the Blue transmitter. On 7 October 1983 Blue was followed for about 6 h as it ascended from 250 m depth to 150 m. At the end of this period the animal resettled to the bottom, after having travelled ~1 km horizontal distance. Although *Nautilus* appear to move mainly on the bottom, there does seem to be some movement within the water column.

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Gradual phyletic evolution at the generic level in early Eocene omomyid primates

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Analysis of dental morphology in over 600 stratigraphically controlled specimens of tarsier-like primates from early Eocene strata in the Bighorn Basin, Wyoming, provides important new data for understanding the tempo and mode of evolution in primates. Here we describe two evolutionary transitions at the generic level in separate lineages of the family Omomyidae. In both lines transformation occurred not only continuously (rather than by abrupt appearance of new morphologies followed by stasis), but also in mosaic fashion, with greater variation in certain characters preceding a shift to another character state. In one lineage this resulted in a significant change in dental adaptation. These data support a gradual model of dental evolution and are inconsistent with the punctuated equilibria model. Together with other reported cases, the data suggest that gradualism is much more prevalent than proponents of punctuated equilibria have argued.

The early Eocene sequence of the Willwood Formation in the Bighorn Basin, Wyoming, is more than 700 m thick, spans about 4 Myr and is among the richest areas for fossil vertebrates in the world. Because of excellent stratigraphical control, samples from these deposits constitute some of the most important data for studies of mammalian evolution[1–12], including the largest and most diverse collection of omomyid primates known from any basin. This collection is now several times larger than the largest sample studied by previous workers, and nearly all specimens are tied into measured stratigraphical sections.

The omomyid samples have been assigned to at least six species in as many as six genera: *Teilhardina americana*, *Tetonoides tenuiculus*, *Tetonius homunculus*, *Pseudotetonius ambiguus*, *Absarokius abbotti* and *Omomys vespertinus*[7,13–20], and several undescribed species (Fig. 1). The systematics of these fossils is still under study, and it is clear that previous understanding of most of them was very incomplete. Our studies indicate that all of these species are valid, but it is debatable how many genera are represented. Here we provide evidence for the transitions between *Tetonius homunculus* and *Pseudotetonius ambiguus* and between *Teil. americana* and *Tetonoides tenuiculus*. Detailed justification will be provided elsewhere for recognition of *Teil. americana* and *P. ambiguus*, the validity of both of which has been questioned[20–22], but for this report, the taxonomy of the fossils is of less consequence than the evolutionary patterns they show.

All omomyids from the Bighorn Basin are conservative in molar morphology, differing consistently only in absolute size or relative breadth. Figure 1 shows that the molars separate to some extent on size alone. *Teilhardina* and *Tetonoides* are about the same size and are significantly smaller than *Tetonius* or *Pseudotetonius*, whereas the latter and *Omomys* are slightly smaller than *Tetonius*, and *Absarokius* is somewhat larger than *Tetonius*. Even so, the latter four taxa overlap substantially in molar size. To distinguish between them, differences in crown morphology, size and configuration of antemolar teeth are more important than molar size. Within each size group (Fig. 1), different morphologies are recognized under the specific names listed above, and our studies demonstrate that each comes from a restricted stratigraphical interval. Precise stratigraphical documentation has, therefore, been indispensable in determining relationships between species.

In the lineage from *Tetonius homunculus* to *P. ambiguus*, the anterior dentition evolved dramatically, while the molars and the last lower premolar (P_4) remained conservative. Here we apply the name *Tetonius homunculus* to the most primitive specimens (see also ref. 19), which have a relatively large, two-rooted P_3, a very small, one-rooted P_2, a moderate sized canine and second incisor (I_2), and an enlarged medial incisor (I_1). All such specimens (stage 1 in Fig. 2) are confined to the 180–190 m interval of the Willwood Formation in the principal study area (central and southern Bighorn Basin). *Tetonius* first appears at about 65 m but its anterior dentition is unknown below 180 m. Not all specimens in this lineage possess P_2; but from the stratigraphy it became clear that P_2 was lost after stage 1, beginning a trend toward progressive compaction and reduction of anterior teeth, except for I_1 which became larger with time. The name *Tetonius ambiguus* was first applied to a specimen showing extreme compression of the anterior dentition[17], but with increasing knowledge of variation in samples allocated to *Tetonius*, *Tetonius ambiguus* was transferred to a new genus, *Pseudotetonius*[14]. *P. ambiguus*, here shown to be the end point of the lineage, is characterized by a much reduced P_3 with one

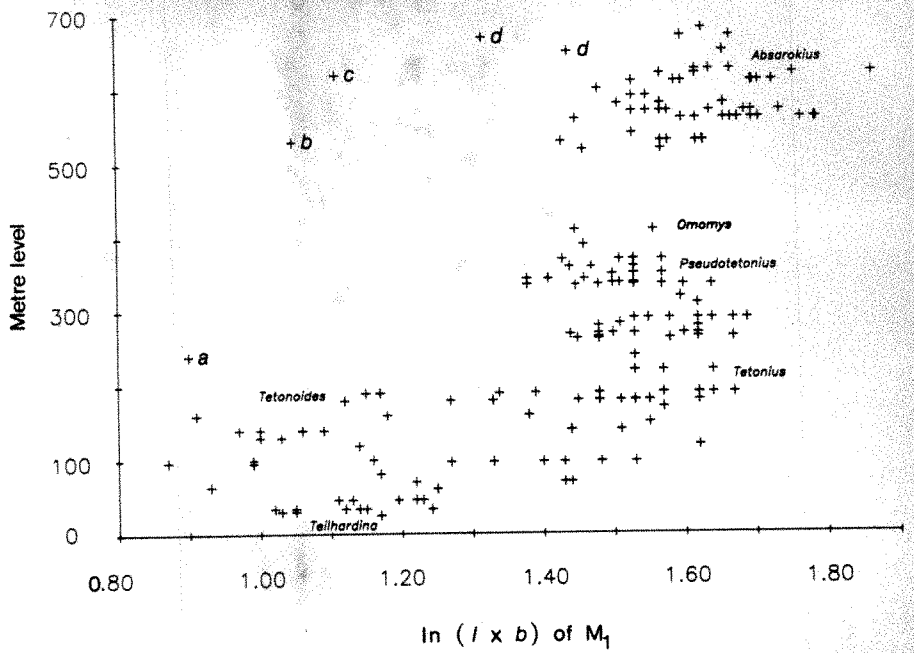


Fig. 1 Stratigraphical distributions of omomyid primates in the central and souther Bighorn Basin (Elk Creek, Fifteenmile Creek and Sand Creek sections). Abscissa show the natural logarithm of crown area (mm^2) of the first lower molar; the ordinate represents the stratigraphical level in Willwood Formation. Some points represent more than one individual. Names are placed near clusters of specimens that can be referred on morphological grounds to the species listed in the text and points situated stratigraphically between them represent evolutionary intermediates. Points *a–d* denote individuals belonging to undescribed taxa. Some taxa overlap considerably in size range of M_1, but their means may differ: for example, mean area of M_1 in *P. ambiguus* is less than in *Tetonius homunculus* and both are smaller than the mean for *A. abbotti*. Plots for other teeth show a similar pattern except that those based on P_3 and M_2 show greater separation between *Tetonius homunculus* and *P. ambiguus*, and that based on P_4 segregates *A. abbotti* more from other samples.

root or coalesced roots, absence of P_2, diminutive canine and I_2, and much enlarged I_1 (stages 4 and 5 in Fig. 2). The dentary anterior to P_4 is much shorter than in *Tetonius homunculus*. This morphology occurs in the 350–390 m interval in the study area, at the top of which *P. ambiguus* apparently became extinct (coinciding with an extinction–immigration event termed biohorizon B^{23}). Intervening strata have revealed morphologically intermediate specimens that record a gradual transition from *Tetonius homunculus* to *P. ambiguus* (Fig. 2).

Of particular interest is the mosaic pattern of change. Modifications that characterize *Pseudotetonius* did not appear synchronously, and certain characters (for example, P_3 root configuration) were more variable during periods of change. P_2 disappeared between stages 1 and 2; P_3 gradually diminished in size throughout the sequence while its two roots became closely appressed and merged into one from stages 2 to 5; the canine and I_2 became gradually smaller up-section; and I_1 enlarged, principally in stages 3 to 5. There was also a slight reduction in mean lengths of P_4 and the lower molars in stages 3–5. The sum of changes during this transition reflect an adaptive

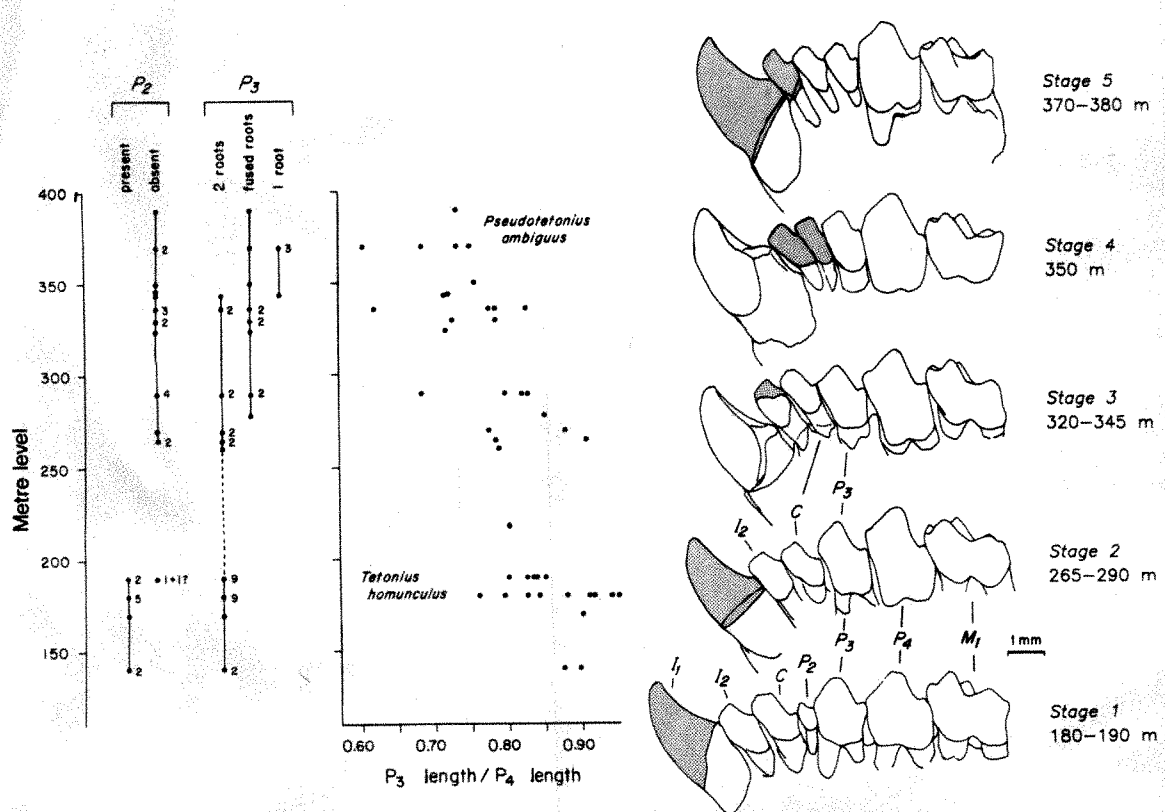


Fig. 2 Evolution of the anterior lower dentition in the *Tetonius homunculus–P. ambiguus* lineage from the central and southern Bighorn Basin. Stages in the transition are arbitrarily delimited by slight morphological differences and/or intervals with little or no data, and each is a composite dentition (M_{2-3} omitted) based on all known material from the indicated interval. Shaded parts are reconstructed from root size and known structure in other stages. The stratigraphical distribution of certain character states is shown on the left; numbers of individuals are indicated except where data points represent single specimens.

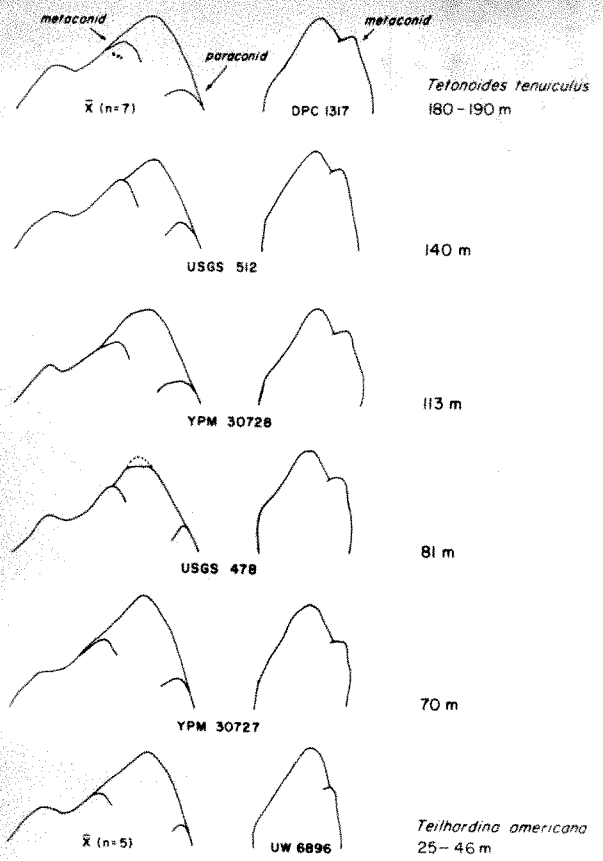


Fig. 3 Transformation of lower fourth premolar (P_4) in the *Teil. americana–Tetonoides tenuiculus* lineage. Left, computer-generated digitized lingual profiles, standardized to the same length so as to indicate only structural differences. Note the progressive enlargement and elevation of the metaconid and the elongation of the paraconid in successively higher strata. Right, camera lucida tracings, at constant magnification, of the back of the P_4 trigonid, showing the increase in size and height of the metaconid as well as increase in absolute breadth. Profiles for *Teil. americana* and *Tetonoides tenuiculus* are means computed from only complete, unworn specimens; other profiles depict individual specimens. Other known specimens from these levels conform to the morphologies shown.

shift to shorter-jawed omomyids with enhanced emphasis of the anterior grasping teeth and declining importance of the teeth just behind them.

Also found is a gradual transition from *Teil. americana* to *Tetonoides tenuiculus*, best documented in the morphology of the posterior lower premolars (P_{3-4}). *Teil. americana* is the oldest and most conservative North American omomyid[13,24] and, as in European *Teilhardina belgica* [clearly its congener (K.D.R., T.M.B. and M. Godinot, in preparation)], the P_3 and P_4 are relatively high-crowned, pointed and bear small, low conids, which are more pronounced on P_4; the cheek teeth are not basally inflated. These features occur only in the lowest 50 m (25–50 m interval) of the study area. *Tetonoides tenuiculus* differs from *Teilhardina* chiefly in having relatively broader and lower cheek teeth and a more molariform P_4, best illustrated by the higher, more salient paraconid and metaconid. Specimens in which this morphology is fully developed come from the 180–190 m interval in the study area. Fossils from intervening strata are intermediate between the two morphologies and become progressively more like *Tetonoides* up-section, the cheek teeth increasing in relative breadth and the paraconid and metaconid increasing in prominence with time (Fig. 3). At no level in the section is there an obvious morphological shift (punctuation); rather, change appears to be continuous and gradual. *Tetonoides tenuiculus* was short-lived in the Bighorn Basin (but a few stratigraphically higher specimens may be related to it: Fig. 1*a–c*), although the genus is known from younger rocks in other basins[25,26].

Both of the phyletic transitions described here are also recorded in samples from the Clark's Fork Basin, 100 km north of the study area. Those samples show the same sequence of changes in both lineages as observed in the study area, thus providing independent confirmation of the patterns reported here.

The tempo and mode of evolutionary change continue to be among the most actively debated issues in evolutionary biology, with particular attention currently focused on punctuational and gradual models of evolution[3,11,12,27–35]. The most convincing evidence for gradual evolution in fossil mammals has been advanced by Gingerich and colleagues[1–6], but these studies concentrate primarily on changes in molar size, and advocates of the punctuated equilibria model largely discount them[11,12]. The transitions in two lineages of omomyid primates detailed here involve a mosaic of continuously changing features that document the gradual evolution of new morphological types of generic rank. Together with Gingerich's examples and other detailed cases of gradual evolution[36–38], our data suggest that phyletic gradualism is not only more common than some would admit but also capable of producing significant adaptive modifications.

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Close genetic linkage between X-linked retinitis pigmentosa and a restriction fragment length polymorphism identified by recombinant DNA probe L1.28

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Retinitis pigmentosa (RP) is a group of retinal degenerations characterized by progressive visual field loss, night blindness and pigmentary retinopathy[1]. Its prevalence is in the region of 1–2 in 5,000 of the general population, making it one of the commoner causes of blindness in early and middle life[2,3]. Although 36–48% of RP patients are isolated cases, the remainder show autosomal dominant, autosomal recessive or X-linked modes of inheritance[4,5]. The X-linked variety (XLRP) is found in 14–22% of RP families in the UK[2,5]. In the present study, X chromosome-specific recombinant DNA probes which can detect restriction fragment length polymorphisms have been used to localize the XLRP gene(s) to a subregion of the X chromosome using linkage analysis. One of the probes, L1.28, has been shown to be closely linked to XLRP in five kindreds, with 95% confidence limits of 0–15 centimorgans (maximum LOD score of 7.89 at a distance of 3 centimorgans). This suggests that the XLRP locus lies on the proximal part of the short arm of the X chromosome. This probe is potentially useful for carrier detection and early diagnosis in about 40% of cases, provided that genetic heterogeneity can be excluded by analysis of further families.

XLRP is one of the most severe clinical forms of RP, with onset in males in the first decade, progressing to blindness by the third or fourth decade[6]. Female carriers show a variable but generally mild degree of visual loss, which occurs much later than in males and is associated with characteristically mild ophthalmoscopic changes in the retinal fundus and abnormalities on visual field, psychophysical and electrophysiological testing[7–9]. Partial manifestation in female carriers is generally attributed to random X-chromosome inactivation leading to unmodified expression of the XLRP allele in a proportion of retinal cells. Clinical and electrophysiological testing may detect only a proportion of carriers and then only at a relatively late stage of the disease. The loci for the Xg blood group and deutan colour blindness are not closely linked to XLRP and are therefore not useful in carrier detection[10,11]. Recently, restriction fragment length polymorphisms (RFLPs) have extended the utility of polymorphic markers in the detection of genes responsible for genetic disorders such as Huntington's chorea and Duchenne muscular dystrophy[12,13].

Families with XLRP were ascertained through ophthalmological genetic clinics in Edinburgh, Birmingham and London. The criteria used for diagnosis and establishment of X-linkage have been described previously[14]. Females at risk showing the ophthalmoscopic changes of carrier status, which were con-

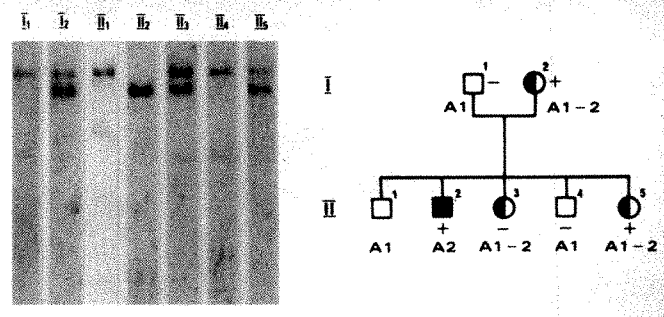


Fig. 1 The segregation of RFLP alleles identified with probe L1.28 in an XLRP family (F21). Carrier females (◐) are shown in generations I (I2) and II (II.3, 5). The mother (I.2) is an obligate heterozygote as she has a severely affected son (II.2, ■) and two carrier daughters. She is also heterozygous for the L1.28 polymorphism, showing the upper (12 kb) and lower (9 kb) bands. The unaffected sons (II.1, 4) have inherited allele A1 (12 kb) while the affected son has inherited allele A2 (9 kb) from the mother, as have the two carrier daughters. $Xg^a(+)$ and $Xg(-)$ blood group alleles are also shown.
Methods: DNA was prepared from 20 ml of whole blood or lymphoblastoid cell lines by the method of Kunkel *et al.*[22] and digested to completion using 5 units of *Taq*I per μg DNA for 20 min at 65 °C. The DNA digests (5 μg per track) were separated by overnight gel electrophoresis in 0.8% agarose, transferred to a nitrocellulose filter by the method of Southern[23] and hybridized for 18 h at 65 °C in 15 ml of 5 × SSC, 5 × Denhardt's solution, 0.1% SDS and 100 μg ml^{-1} denatured salmon sperm DNA. The L1.28 plasmid DNA was labelled by nick-translation with ^{32}P-dTTP (specific activity ~2×10^8 c.p.m. μg^{-1}), without excising the insert. The probe concentration in each filter hybridization was 10 ng ml^{-1} (3×10^7 c.p.m.). The filters were washed at room temperature in 2 × SSC for 20 min, followed by three washes of 20 min each in 0.1 × SSC, 0.1% SDS at 60 °C, then autoradiographed on Fuji film at −70 °C for 3–7 days using an intensifier screen.

Table 1 Analysis of linkage between XLRP, Xg and L1.28

Recombination fraction, Θ	XLRP −F15 $n=4$	L1.28 F15 alone $n=1$	XLRP +F15 $n=5$	Xg XLRP +F15 $n=8$
0.00	3.45	−∞	−∞	−∞
0.05	3.15	4.65	7.79	−6.81
0.10	2.82	4.45	7.27	−4.00
0.20	2.11	3.69	5.80	−1.65
0.30	1.33	2.69	4.02	−0.63
0.40	0.57	1.47	2.03	−0.16
Θ_{max}	0.00	0.04	0.03	0.50
LOD_{max} *	3.45	4.65	7.89	0.00
Θ_{mean}	0.08	0.10	0.06	0.47
Range (cM)	0–27	2–25	0–15	38–200
Odds (Θ < 0.3)	79:1	187:1	15,000:1	0.019:1
Odds (Θ < 0.2)	20:1	19:1	400:1	0.001:1

LOD scores are shown for different values of the recombination fraction (Θ) in XLRP families excluding F15 (−F15), including F15 (+F15) and in F15 alone. Maximum likelihood values of Θ (Θ_{max}) and the corresponding LOD scores (LOD_{max}) are shown together with the mean Θ values, the latter being a value more appropriate to genetic counselling[20]. A probability distribution for the recombination fraction was calculated from the likelihood curve (calculated at 51 values of Θ) and the linear prior probability for two unknown loci[21]. The Kosambi mapping function was used and the X chromosome was assumed to be 200 centimorgans long. The 95% confidence limits were estimated by excluding 2.5% of the area under the curve at each tail. The 'odds of linkage' were estimated by comparing the area under the curve at values of Θ less than 0.3 or 0.2 with that under the remainder of the curve. The number of informative kindreds is indicated (n).
* The LOD score is the $\log_{10}$ of the likelihood ratio, which is calculated by dividing the likelihood of observing the family data, for any value of Θ, by the likelihood if Θ is 0.5 (that is, random assortment or infinite separation of two loci).

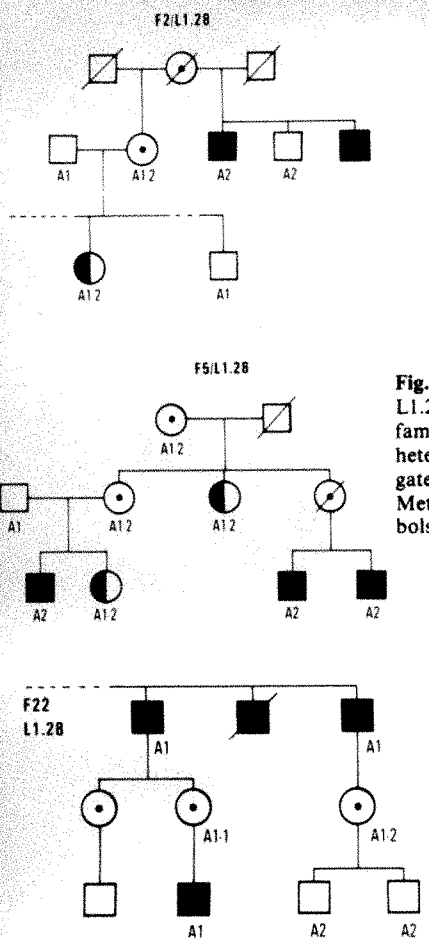


Fig. 2 Pedigrees and L1.28 alleles in XLRP families 2, 5 and 22. XLRP heterozygotes: ⊙, obligate; ◐, on testing. Methods and other symbols as described in Fig. 1 legend.

firmed by psychophysical and electrophysiological tests, were taken to be XLRP heterozygotes; otherwise the genetic status of females was not inferred unless the pedigree structure showed them to be obligate heterozygotes. Blood samples were obtained from 250 members of 24 XLRP families and lymphoblastoid cell lines were raised from obligate heterozygotes and individuals over 60 yr old.

Estimates of the genetic length of the X chromosome (150–250 centimorgans)[15] suggest that it should be possible to localize the XLRP gene with 10 suitably spaced X chromosome-specific recombinant DNA probes capable of identifying RFLPs. Three such probes assigned to the X chromosome short arm, each of which detects RFLPs with the same restriction endonuclease, *Taq*I, have been used so far; one of these, L1.28, was isolated from a human genomic library prepared from the placental DNA of a single individual by Pearson *et al.*[16]. The L1.28 probe is a single-copy X chromosome-specific DNA sequence, 1.25 kilobases (kb) long, that has been inserted in the *Eco*RI site of plasmid pBR322. It has been regionally assigned to Xp11.0-p11.3 by using somatic cell hybrids[16]. This probe identifies two RFLP alleles with the enzyme *Taq*I: A1, which is 12 kb long and A2, which is 9 kb long. These alleles occur at frequencies of 0.68 and 0.32 respectively in the Dutch population, on the basis of an analysis of 40 chromosomes. Digestion of DNAs from normal individuals using nine other restriction enzymes did not identify RFLP with L1.28 (ref. 16). In addition, 50 X chromosomes from 29 normal individuals have been analysed in Edinburgh and the frequencies of A1 and A2 were found to be 0.74 and 0.26. Overall, this suggests a heterozygote frequency of 0.41 (95% confidence limits 0.33–0.47).

Panels of DNA from XLRP obligate heterozygotes previously digested with *Taq*I were screened for RFLP heterozygosity using probe L1.28. Nine individuals, from five kindreds, were informative for the L1.28 polymorphism, out of a total of 28 obligate heterozygotes screened. The segregation of L1.28 alleles in one XLRP sibship is shown in Fig. 1. Linkage analysis was done using the computer program LIPED[17] and checked by hand.

In four of the kindreds, each with typical XLRP, there were no definite recombinants (Figs 1, 2) and the value of the recombination fraction (Θ), at which the likelihood is a maximum, is zero with a LOD score of 3.45 (Table 1). The fifth kindred (F15) was described by Hoare as having X-linked choroidoretinal dystrophy[18]. After review and subsequent examination, the consensus of opinion (G. W. Hoare, A.C.B. and B. S. Jay) is that the disorder in this kindred is within the clinical spectrum of XLRP. However, as the possibility of a second locus cannot be excluded, the scores for this kindred are presented both separately and together with the other kindreds (Table 1). Only one female in F15 (III.13), identified as a carrier on testing, was found to be a definite recombinant; the remainder were non-recombinant (Fig. 3). In F15, the maximum likelihood value of Θ is 0.04 at a LOD score of 4.65. The linkage data are therefore consistent with the F15 locus being identical with, or close to, that in the other kindreds. When the data from all the kindreds are combined, the maximum likelihood value of Θ is 0.03, corresponding to a distance of 3 centimorgans, at a LOD score of 7.89. The 95% confidence limits are 0–15 centimorgans and the odds of linkage at Θ values less than 0.3 are 15,000:1 (see Table 1).

Xg blood group data have been analysed in eight kindreds typed by Dr R. Sanger and Dr P. Tippett of the MRC Blood Group Unit, London (Table 1). Close linkage is excluded and

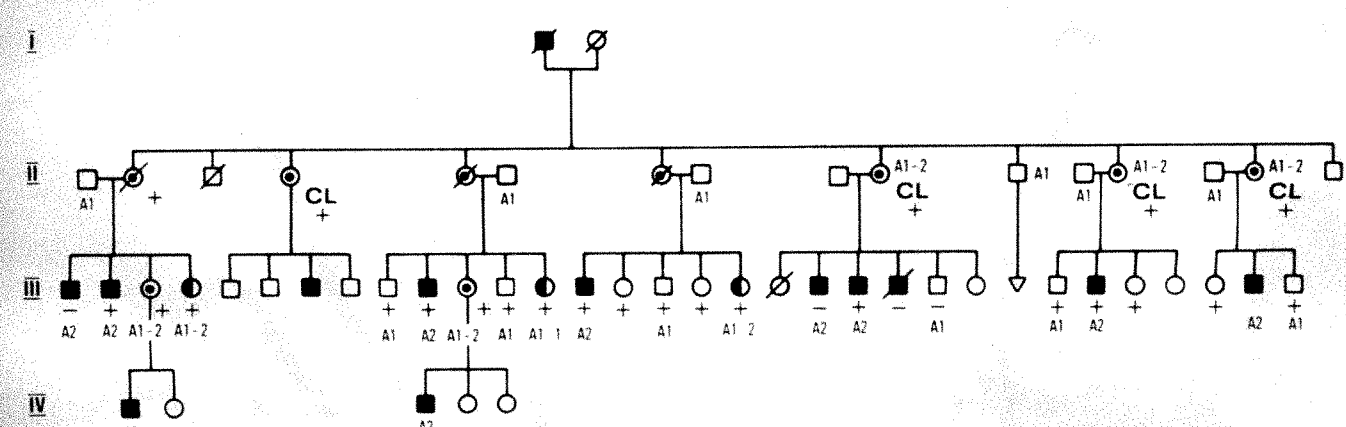


Fig. 3 F15 pedigree with L1.28 and Xg blood group results. This family is highly informative for the L1.28 polymorphism. All of the obligate heterozygotes available in generation II are also heterozygous for the RFLP and, with one exception, all XLRP hemizygotes (■) and heterozygotes (⊙, obligate; ◐, on testing) have inherited allele A2. The exception is III.13, who is homozygous for allele A1 and an XLRP carrier on testing; she is therefore a recombinant. L1.28 results of individuals whose XLRP status is uncertain have not been included in the pedigree. CL denotes that a lymphoblastoid cell line has been raised from that individual. $Xg^a(+)$ and $Xg(-)$ alleles are also shown. At least three recombinants between Xg and XLRP can be seen.

the 95% confidence limits for the genetic distance between the XLRP and Xg loci are 38–200 centimorgans. The Xg locus has been assigned to Xp22.3-pter[19] and the genetic length of the short arm of the X chromosome (Xp) is in the region of 60–90 centimorgans[15], so that the assignment of XLRP to within 15 centimorgans of L1.28 (assigned to Xp11.0-p11.3) is consistent with known distances on Xp. Recent evidence from somatic cell hybrids shows that the L1.28 locus is close to Xp11.3 (P.L.P. *et al.*, unpublished), so that while the precise relationship between physical and genetic maps in this region has yet to be established, it is likely that the XLRP locus is on the proximal part of Xp.

The finding of close genetic linkage between XLRP and the L1.28/*Taq*I polymorphism suggests that this could provide a useful means of carrier detection or prenatal diagnosis. However, more XLRP families informative for this RFLP must be examined to exclude significant genetic heterogeneity. Further work is also required to make more families informative for L1.28 and to map XLRP in relation to other neighbouring RFLPs.

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Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines

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The recent availability in culture of embryo-derived pluripotential cells which exhibit both a normal karyotype and a high differentiative ability[1–3] has encouraged us to assess the potential of these cells to form functional germ cells following their incorporation into chimaeric mice. We report here the results of blastocyst injection studies using three independently isolated XY embryo-derived cell lines (EK.CP1, EK.CC1.1 and EKCC1.2) which produce a very high proportion (>50%) of live-born animals that are overtly chimaeric. Seven chimaeric male mice, derived from these three lines, have, so far, proved to be functional germ-line chimaeras.

Although teratocarcinoma-derived embryonal carcinoma (EC) cells have been used to construct chimaeras, they have rarely realized the developmental potential demonstrated by embryonic inner cell mass cells used in a similar combination[4]. Indeed, when introduced into the early embryo many EC cell lines show a low rate of colonization and/or a restricted pattern of differentiation[5], and many chimaeras develop tumours both pre- and postnatally[4].

We have recently demonstrated that over 15 of our fertilized and parthenogenetically activated[6] embryo-derived (EK) cell lines of both XX and XY sex chromosome constitutions form normal chimaeras with high efficiency (in preparation). In this study we decided to use fertilized embryo-derived pluripotential XY cell lines, which, unlike most of their tumour-derived EC cell counterparts, readily differentiate *in vitro* and possess a normal euploid karyotype[3], an essential prerequisite for the formation of viable gametes. We now present data which clearly demonstrate that such cell lines are capable of forming functional germ-line chimaeras.

Table 1 gives the results of the blastocyst injection experiments and Table 2 the results of the test breeding studies. Figure 1 shows six of the phenotypically male chimaeras which transmitted functional spermatozoa derived from the introduced cells.

It would obviously be desirable to incorporate specific functional genes into the mouse genome. While direct transformation

Table 1 Rates of construction of chimaeras

Cell line	No. injected	No. born (%)	No. chimaeric (%)	Chimaeras Male	Chimaeras Female	Chimaeras ND	Males Set up	Males Bred	No. of germ-line chimaeras
EK.CP1	254	167 (66)	74 (44)	40	27	7	23	20	4
EK.CC1.1	69	52 (75)	31 (60)	21	10	0	13	8	1
EK.CC1.2	160	111 (70)	63 (57)	50	13	0	21	7	2
			Total:	111	50			35	7 (20%)

The EK.CP1, EK.CC1.1 and EK.CC1.2 cell lines were isolated from delayed blastocysts[1]. All of these lines are characterized by the possession of black and agouti coat colour markers. The EK.CP1 cell line was derived from a 129/Sv//Ev strain mouse and is homozygous for the *GPI-1*^{a} allele at the *GPI-1* (glucose phosphate isomerase-1) locus. EK.CC1.1 and EK.CC1.2 were derived from a substrain of 129/Sv//Ev which is homozygous for the *GPI-1*^{c} allele at the *GPI-1* locus. All three of these lines possess a normal euploid XY chromosome constitution and have been maintained entirely *in vitro* on feeder layers of inactivated fibroblasts. The breeding data available for some males are incomplete; these might yet prove to have a low-level culture-derived germ-line component. There is a very evident distortion of the sex ratio in the live-born chimaeras—this deviation in favour of males is highly significant (χ^2, $P < 0.001$) and probably reflects the conversion of a number of 'host' female embryos to male chimaeras. ND, not determined.

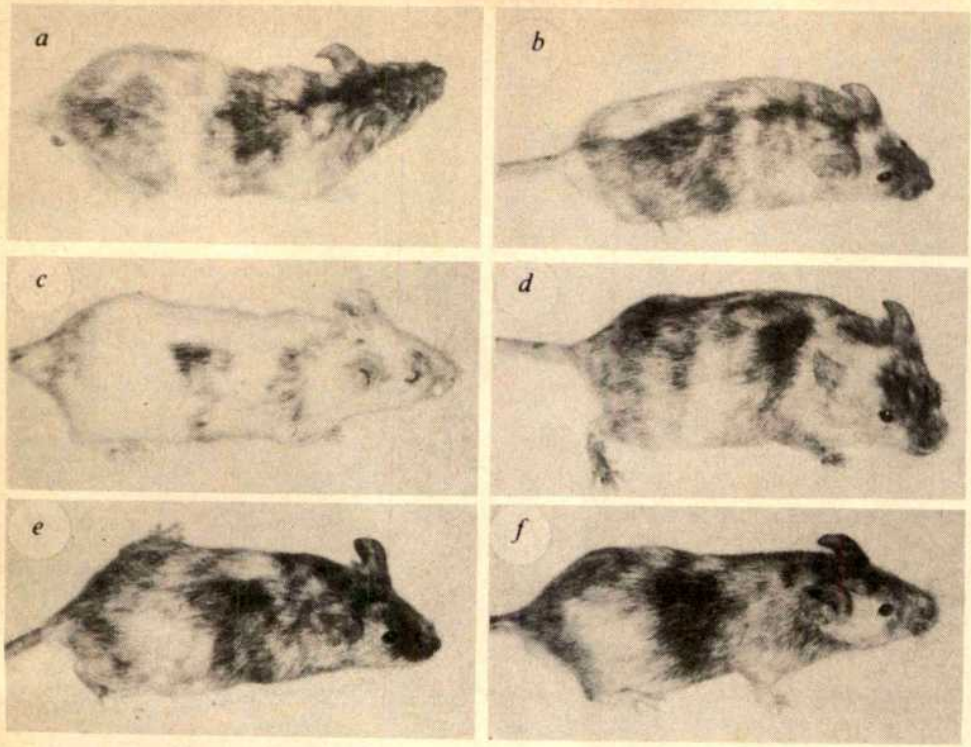


Fig. 1 Six of the seven germ-line chimaeras described in Tables 1 and 2. *a*, CP1.3; *b*, CP1.5; *c*, CP1.11; *d*, CC1.1.3; *e*, CC1.2.6; *f*, CC1.2.8. Between 8 and 12 embryo-derived cells were introduced into the blastocoelic cavity of host-fertilized blastocysts homozygous for the recessive albino locus. The blastocysts were then allowed to re-expand and were subsequently transferred to the uterine lumen of recipients on the third day of pseudopregnancy. All the conceptuses were allowed to develop to term, and live-born animals were scored for the presence of eye and coat pigmentation at or shortly after birth.

of the zygote has recently shown much promise[7–13], an alternative approach is the construction of individuals between 'modified' pluripotential cells and normal embryos. This second approach has the advantage that cultured cells are accessible for genetic manipulation, characterization and selection before their incorporation *in vivo*. Several EC cell lines have been selected *in vitro* to carry specific mutations or additional intact 'foreign' chromosomes, and have subsequently been incorporated into chimaeric individuals[14–17]. It is, however, a necessary prerequisite to demonstrate that germ-line chimaerism can be reliably achieved. Published rates of construction of germ-line chimaeras using EC cell lines have been disappointingly poor[18,19]. There has been a report of a single XX cell line, maintained completely *in vitro*, from which two germ-line females have been obtained. In addition to a low rate of chimaera formation (13%), these females produced only four progeny having an EC-derived component[20,21].

Table 2 Breeding data from the seven functional germ-line chimaeras

Male chimaera	No. of litters	No. of offspring	No. albino	No. black agouti
CP1.3	10	78	0	78
CP1.5	16	170	163	7
CP1.11	2	23	22	1
CP1.34	1	5	0	5
CC1.1.3	1	12	0	12
CC1.2.6	1	13	0	13
CC1.2.8	1	11	0	11

Phenotypically male chimaeras were caged with successive virgin CFLP females homozygous for the *GPI-1*^{b} allele and the albino locus. Resulting litters were scored for the dominant black and agouti phenotypes. GPI analysis of blood samples taken from all the black agouti offspring has shown the predicted *GPI-1*^{a}*1*^{b} (EK.CP1) or *GPI-1*^{b}*1*^{c} (EK.CC1.1 and EK.CC1.2) genotype, thus verifying the inheritance of these culture-maintained lines. The chimaeras which appear to be breeding from their XY culture-derived component alone are probably sexual mosaics, although phenotypically they are normal males, as any XX germ cells would not be capable of forming functional spermatozoa.

Clearly, embryo-derived stem cells seem to be particularly efficient at recolonizing the early embryo. This feature, together with the availability of XY lines such as those described here, now allows the routine construction of chimaeric males which are capable of transmitting culture-derived genomes to a potentially limitless number of offspring, and confirms our previous contention of the normality of the genome in these stem cell lines[22].

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Note added in proof: A further four males described in Table 1 have proved to be germ-line chimaeras (three from the CC1.2 and one from the CC1.1 cell lines).

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Spinal opiates affect sexual behaviour in rats

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Enkephalin-containing cells in the substantia gelatinosa of the spinal cord[1] participate in the neural processing of painful stimuli[2,3]. Spinal opiates may also be involved in the perception of sensory stimuli related to reproduction, as probing of the uterine cervix stimulates sexual behaviour in rats[4] and also induces an analgesia[5] which is partially dependent on opiate receptor mechanisms in the spinal cord[6–8]. We now report that the intrathecal injection of morphine into female ovariectomized rats pretreated with oestradiol benzoate and progesterone inhibits sexual receptivity while injection of the opiate receptor antagonist naloxone enhances it. Similarly, intrathecal injection of morphine increases while injection of naloxone decreases the number of intromissions before ejaculation in male rat. Intraperitoneal injection of morphine or naloxone has no behavioural effect. Recent studies have suggested that brain opiates are involved in the control of sexual behaviour[9–11]. The results reported here indicate that sexual behaviour may also be influenced by spinal opiates.

Female Wistar rats (200–220 g) were ovariectomized and 1 week later a polyethylene catheter was inserted into the subarachnoidal space of the lumbosacral spinal cord as described elsewhere[12]. One week later the rats were injected with oestradiol benzoate and progesterone and tested individually with male rats. All females showed lordosis (concave back flexion, lateral tail deviation and neck extension) to all mounts and were thereafter divided into four groups and injected intrathecally (i.t.) with the opiate receptor agonist morphine in doses which produce moderate analgesia but do not disturb motor function[13,14]. The number of lordosis responses to 10 mounts by the male was recorded 15 and 60 min after the morphine injection and a lordosis quotient was calculated as: (number of lordosis responses) × 10.

Fourteen ovariectomized rats, pretreated with oestradiol and progesterone, were divided into three groups and injected intraperitoneally (i.p.) or i.t. with morphine. One group given morphine i.t. was injected i.p. with the opiate receptor antagonist naloxone and the other group received an i.p. injection of NaCl. The rats were tested with males 60 min after the morphine injection and lordosis quotients were calculated when the males had mounted 10 times.

Sixteen ovariectomized rats, which showed an overall mean ± s.e.m. lordosis quotient of 43.6 ± 10.5 after pretreatment with a low dose of oestradiol and progesterone were divided into three groups and injected with naloxone i.t., in doses which produce moderate hyperalgesia[15,16], or i.p., or with NaCl i.t. The rats were tested with males 10 min after the naloxone injection.

Sexually experienced Wistar male rats (300–350 g) were implanted with i.t. catheters and tested 1 week later for sexual behaviour. A sexually receptive female was introduced to each male and the number of mounts with and without intromission and the latency to the first intromission, the interval between the first intromission and ejaculation and the interval between ejaculation and the next intromission were recorded. Two days later the rats were divided into four groups and injected i.t. with NaCl, naloxone, morphine or morphine + naloxone i.p. and tested for sexual behaviour according to the same time schedule as used with the females. Three other groups of males were injected i.p. with NaCl, naloxone or morphine and tested for sexual behaviour as above.

Figure 1*a* shows that i.t. injection of morphine suppressed sexual receptivity over time. Between-group comparisons showed that morphine caused a dose-dependent inhibition of lordosis both 15 and 60 min after i.t. injection. As little as 1 μg

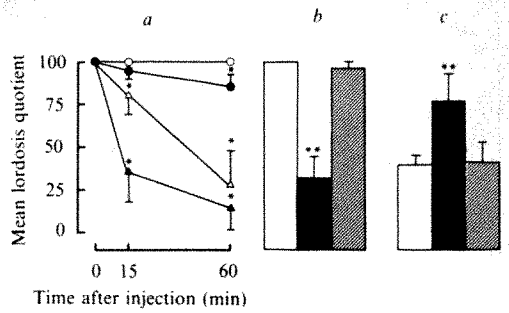


Fig. 1 *a*, Effect of intrathecal (i.t.) injection of 0 (○), 1 (●), 5 (△) or 10 (▲) μg of morphine on sexual receptivity (mean ± s.e.m. lordosis quotient) induced by 10 μg oestradiol benzoate (48 h) and 0.5 mg progesterone (6 h) before the morphine injection. *b*, Effect of intraperitoneal (i.p., open bar) or i.t. (black bar) injection of 5 μg morphine 60 min before testing and of i.t. injection of 5 μg morphine 60 min before testing in combination with 400 μg naloxone i.p. (cross-hatched bar) given 10 min before testing on sexual receptivity induced as in *a*. *c*, Effect of i.p. (open bar) or i.t. (black bar) injection of 50 μg naloxone or 0.9% NaCl i.t. (cross-hatched bar) given 10 min before testing on sexual receptivity induced by 0.8 μg oestradiol (48 h) and 0.5 mg progesterone (6 h) before the naloxone injection. Oestradiol and progesterone (Sigma) were dissolved in 0.1 ml sesame oil and injected subcutaneously. Morphine chloride (Apoteksbolaget) was dissolved in 10 μl 0.9% NaCl and injected i.t. through a Clay Adams PE10 polyethylene catheter or in 0.5 ml NaCl i.p. The i.t. catheter was flushed with 10 μl NaCl after the injection. Naloxone hydrochloride (Endo Laboratories) was dissolved and injected as the morphine. * Significantly different from NaCl-injected controls or ** i.p. morphine-injected (*b*) or i.p. naloxone-injected (*c*) rats; *P* at least <0.05 Mann–Whitney *U*-test after one-way analysis of variance. There were four to six rats per group.

morphine significantly inhibited lordosis behaviour 60 min after injection. Rats given an i.t. injection of morphine showed significantly lower lordosis quotients than rats given morphine i.p., while i.p. injection of naloxone reversed the effect of the i.t. morphine injection (Fig. 1*b*). Figure 1*c* shows that an i.t., but not i.p., injection of naloxone facilitated sexual receptivity in rats given a low dose of oestradiol and progesterone.

Males given naloxone i.t. showed fewer and males given morphine i.t. showed more intromissions before ejaculation than males treated with NaCl i.t. (Fig. 2*a*). The effect of morphine was reversed by an i.p. injection of naloxone (Fig. 2*a*). None of the other parameters of sexual behaviour was changed by the i.t. injections (data not shown). Intraperitoneal injections of either naloxone or morphine had no effect on intromission frequency (Fig. 2*b*).

In the female rat, spinal segments L1 to S1 are important for processing sensory stimuli provided by the male during copulation[17]. Denervation of peripheral skin[18] or transection of the spinal cord at levels receiving cutaneous information during copulation[19] reduces the intensity of sexual behaviour in female rats. Injection of morphine in the subarachnoidal space surrounding these spinal segments produced a dose-dependent inhibition of sexual receptivity in female rats in our study, and this effect was reversed by naloxone. Also, an i.t. naloxone injection facilitated the behaviour of female rats made partially receptive by injection of low doses of ovarian hormones. Since no behavioural effects were noted when either morphine or naloxone was administered at a site distant from the spinal cord, we suggest that one function of dorsal horn opiates in female rats is to modulate sensory stimulation provided by the male during copulation. Thus, stimulation of the uterine cervix of female rats occurs naturally during sexual interactions in the rat[20] and it is tempting to speculate that the ensuing analgesia, which is partially opiate dependent[5], may reflect a copulation-induced release of enkephalins in the dorsal horns of the spinal cord. This, in turn, may underlie such a physiologically relevant phenomenon as copulation-induced inhibition of behavioural oestrus in the female rat[21].

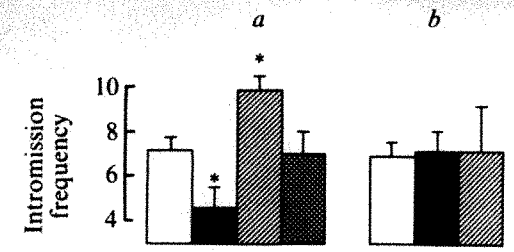


Fig. 2 Effect of intrathecal (*a*) and intraperitoneal (*b*) injection of 0.9% NaCl (open bars), 50 μg naloxone (black bars), 5 μg morphine (cross-hatched bars) or 5 μg morphine in combination with 400 μg naloxone i.p. (stippled bar) on intromission frequency (mean ± s.e.m. number of intromissions before ejaculation) in male rats tested with sexually receptive female rats. Naloxone was given 10 min and morphine was given 60 min before testing. * Significantly different from NaCl-injected controls; $P < 0.05$ Mann–Whitney *U*-test after one-way analysis of variance. There were four or five rats per group.

During copulation, male rats show multiple intromissions, which serve to facilitate ejaculation[22]. Anaesthesia of the penis[23] or section of the dorsal penile nerve[24] prevents the display of intromission and ejaculation. Thus, sensory stimulation of the penis is required for normal copulation in male rats. It seems likely that spinal opiates may serve a function in coding the sensory information value of each intromission as an i.t. naloxone injection decreased the number of intromissions shown by males before ejaculation and an i.t. morphine injection produced a naloxone-reversible increase in intromission frequency. Naloxone or morphine injections had no behavioural effects if given i.p. Sensory information from the penis is carried by the dorsal penile nerve, the cell bodies of which are located in dorsal root ganglion L6[25]. These cells have extensive collateral projections to segments T12–S3 in the substantia gelatinosa of the spinal cord[25], that is, the segments around which the i.t. injections were made in our study.

Recent work on the physiological role of spinal opiates have concentrated exclusively on their involvement in the perception of pain[1,2]. The present results suggest that enkephalins in the spinal cord may participate in the coding of a broader range of sensory information.

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A vasopressin-like peptide in the mammalian sympathetic nervous system

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Vasopressin was among the first mammalian hormonal peptides to be identified and to have its structure determined[1]. Its only undisputed physiological role is as a circulating neurohypophyseal antidiuretic hormone. Other notable effects of vasopressin on peripheral tissues include contraction of vascular smooth muscle, leading to elevation of blood pressure, and activation of glycogenolysis in liver. It has long been clear that vascular smooth muscle and hepatocytes are relatively insensitive to the low concentrations of vasopressin normally present in the circulation, and the physiological significance of their responses has therefore been in doubt. We now report that a new bioactive and immunoreactive vasopressin-like peptide (VLP) is widely distributed in the sympathetic nervous system of mammals, both in the principal noradrenergic neurones of ganglia and in nerve fibres innervating peripheral tissues. In addition to other peptides described in the mammalian sympathetic nervous system[2], VLP must be considered as a possible mediator of the non-adrenergic responses to sympathetic activation. Moreover, many of the effects previously attributed to circulating vasopressin may be neurally evoked.

Using several antisera having high specificity for arginine-vasopressin (AVP, Table 1 legend), significant quantities of VLP were detected in rat superior cervical and coeliac ganglia (Table 1). The levels detected in the coeliac ganglia were one-quarter those found in the superior cervical ganglia, suggesting that there may be some rostral–caudal differences in the levels of VLP along the sympathetic chain, as has been found for vasoactive intestinal polypeptide (VIP)[3]. Detectable levels of oxytocin-like immunoreactivity were also found by using an oxytocin-specific radioimmunoassay, but the levels were about one-tenth those found for VLP (Table 1). The apparent VLP levels in rat superior cervical ganglia are comparable to the levels of substance P-like immunoreactivity expressed after explant culture of the ganglia[4].

The Brattleboro rat, a mutant of the Long–Evans strain, lacks AVP and consequently proved an important tool in vasopressin research[5]. Specific radioimmunoassays demonstrated that the superior cervical ganglia of Brattleboro rats had significant levels of both vasopressin-like and oxytocin-like immunoreactivity, although the levels of the former were 40% of those measured in Long–Evans controls (Table 1).

Gel filtration established that VLP migrates as a single immunoreactive peak with a higher apparent molecular weight than authentic vasopressin (Fig. 1). Because VLP was extracted from pooled ganglia for gel filtration experiments, it should be considered that the material present at nerve endings may be a processing product of VLP detected in ganglia. The oxytocin-like immunoreactivity co-migrated with VLP, suggesting that the oxytocin and vasopressin antisera recognized a common peptide. Ganglion extracts contained no authentic AVP on analysis by HPLC.

The cross-reactivity of VLP with some, but not all, AVP and oxytocin antisera permitted an investigation of its distribution

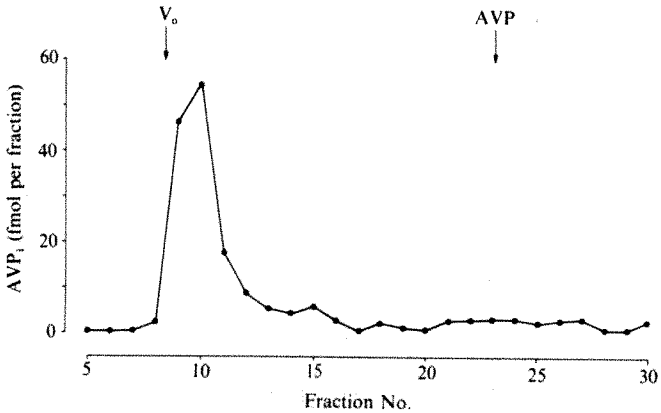


Fig. 1 Elution profile of arginine-vasopressin immunoreactivity from extracts of rat superior cervical ganglia on Sephadex G-25. Extracts were prepared by homogenizing pooled, frozen ganglia from 10 Sprague–Dawley rats in 0.5 ml 50 mM HCl with an ultrasonic disintigrator (MSE Soniprep 150). The homogenate was extracted through acidified octadecasilylsilica columns (Table 1 legend) and resuspended in 1 ml 0.1 M Tris-HCl buffer (*p*H 7.4). This extract was applied to a 1.0×30 cm Sephadex G-25 (fine) column equilibrated in the same buffer. The column was run in this buffer at 45 ml per h at room temperature and 1-ml samples were collected. Aliquots were assayed for arginine-vasopressin immunoreactivity by the technique described in Table 1 legend. Recovery was 70% of that in the applied sample. The void volume (V_0) and the elution position of authentic arginine-vasopressin (AVP) are indicated by arrows. Aliquots were also assayed for oxytocin immunoreactivity, which co-migrated with the AVP-immunoreactivity (AVP_I).

Table 1 Levels of vasopressin- and oxytocin-like immunoreactivity in rat sympathetic ganglia, and comparison with published concentrations of some other immunoreactive neuropeptides in rat sympathetic ganglia

Immunoreactive peptide	Superior cervical ganglion (fmol per ganglion) Normal Long–Evans rat	Superior cervical ganglion (fmol per ganglion) Brattleboro rat	Coeliac ganglion (fmol per ganglion) Normal Long–Evans rat
Vasopressin-like	211 ± 26.5* (12)	85 ± 11.5* (12)	58.2 ± 8.8* (8)
Oxytocin-like	23 ± 0.8* (14)	25 ± 1.6* (13)	ND
	Normal Sprague–Dawley rat		Normal Sprague–Dawley rat
Substance P-like	30–40 (ref. 15)		270 ± 30 (ref.15) 180 ± 40 (ref. 16)
Bombesin-like	ND		3,900 ± 800 (ref. 16)

* Although the recorded concentrations of vasopressin- and oxytocin-like immunoreactivity are based on standards of arginine-vasopressin and oxytocin, the peptide present in ganglia is neither of these (see the text and Fig. 1) and these assays are therefore likely to underestimate the true concentration of ganglionic VLP. The values given are means ± s.e.m. (number of ganglia analysed). ND, not determined. Rats were anaesthetized with sodium pentobarbitone (72 mg per kg) and the superior cervical and coeliac ganglia were dissected and frozen. Each ganglion was then suspended in 0.5 ml 50 mM HCl, homogenized using an ultrasonic homogenizer (MSE Soniprep 150), and centrifuged for 5 min at 11,600*g*. The supernatant was decanted and neutralized with an equal volume of 0.1 M Tris HCl + 10 μl 1 M NaOH. A 0.1-ml aliquot of the neutralized homogenate was added to 1 ml 0.1 M HCO_2H, and passed through an acidified octadecasilylsilica column (C_{18} Sep-Pak; Waters Associates). The column was washed with 3 ml 0.1 M HCO_2H and eluted with 3 ml 95% methanol/5% 0.1 M HCO_2H. The eluate was dried *in vacuo*, resuspended in 0.5 ml assay buffer, and assayed in duplicate by a modification of the technique of Lightman and Forsling[17] at three serial dilutions. In brief, ~4,000 c.p.m. of ^{125}I-arginine-vasopressin tracer (NEN, 1,820 Ci per g) was added and samples were incubated for 48 h at 4 °C. Free and bound tracer were separated by ethanol precipitation, and the bound fraction was counted by a γ-counter. The anti-vasopressin antiserum used here was kindly provided by Stefan Lundin (Ferring Pharmaceuticals). Its cross-reactivity with oxytocin is 0.1%, with lysine-vasopressin 10%, and with desamino-D-arginine vasopressin 0.01%.

by immunocytochemical techniques. Using immunofluorescence, superior cervical, coeliac and thoracic ganglia from two mammalian species (rat, macaque monkey) revealed staining of large neurones in the ganglia (Fig. 2). Because it seemed likely that this staining was restricted to noradrenergic principal neurones, sections of superior cervical ganglia were stained first for VLP and subsequently for dopamine-β-hydroxylase (DBH). All VLP-positive neurones were also stained by anti-DBH, although it is not clear whether all DBH-positive neurones are VLP-positive. It is clear, however, that this pattern is not specific to any particular ganglion or species; it appears to be widespread in the sympathetic nervous system. In some sections, a plexus of nerve fibres could be detected (Fig. 2*c*) surrounding the large neurones. Because the plexus stains for both VLP and DBH, it seems likely that it arises from postganglionic neurones, although its precise anatomical origin is unknown.

Sympathetically innervated tissues were examined for peripheral fibres that might correspond to the projections of the VLP-positive neurones. In liver and kidney, two known targets of vasopressin, numerous immunoreactive fibres were detected. In the hilar region of the liver, VLP-positive nerve fibres were found running in the smooth muscle layers of the hepatic blood vessels (Fig. 3*b*), but none was found in apposition to the bile ducts. Similarly, blood vessels of the kidney, particularly the renal artery (Fig. 3*a*), showed a high density of immunoreactive fibres. In the kidney, fibres were also found in a variety of other structures, for example, in the vicinity of collecting ducts. This is of considerable interest, as vasopressin exerts more than one action on the kidney. A detailed investigation of the renal VLP-positive fibres at both the light and electron microscope level may reveal unexpected possibilities for direct neural control. Similar VLP-positive fibres have been observed in other sympathetically innervated peripheral organs, such as rat submandibular gland.

Because activation of V_1 vasopressin receptors in liver and artery stimulates inositol lipid breakdown[6], we examined inositol lipid metabolism in rat sympathetic ganglia in order to detect functional V_1 receptors. Rat superior cervical ganglia were stimulated with AVP after incubation with ^{3}H-inositol, and the production of inositol phosphates from inositol lipids was measured[7]. The response was rapid, with detectable accumulation of inositol bis- and trisphosphates within 15 s (Table 2*a*), suggesting primary breakdown of phosphatidylinositol 4,5-bisphosphate, as in liver[8]. Stimulated inositol lipid breakdown is also a response of these ganglia to muscarinic cholinergic stimulation, but the response to AVP is several times larger[9]. The half-maximal response to AVP occurred at ~30 nM AVP[9], and was blocked by a specific V_1-receptor antagonist (Table 2*b*). The AVP-stimulated accumulation of inositol phosphates was unaffected by a reduction in extracellular Ca^{2+} concentration, suggesting that it is a primary receptor-coupled event rather than a secondary response to another mediator released from AVP-sensitive neurones. Ganglia showed little or no change in cyclic AMP content when exposed to AVP, indicating that they possess few, if any, functional V_2 receptors. The matching of the biochemical responses in ganglia with those in sympathetic target tissues suggests that this is a coordinated system in which VLP may be the preferred endogenous stimulant of V_1 responses.

To test the biological actions of VLP, a sympathetic ganglion extract was freed of catecholamines by adsorption to a miniature reverse-phase column. When injected intravenously into alcohol-anaesthetized, water-loaded rats, the extract had a marked antidiuretic effect with no effect on blood pressure. Thus, although VLP shares at least one biological action with AVP, the relative potencies at V_1 and V_2 receptors cannot yet be assessed.

The simplest interpretation of the data is that VLP is a novel neuropeptide in postganglionic sympathetic neurones that contain and secrete noradrenaline. In this regard, it is important to establish the relationships between VLP and other neuropeptides detected in mammalian sympathetic ganglia. Only neuropeptide Y appears to have a similar distribution to that of VLP[10].

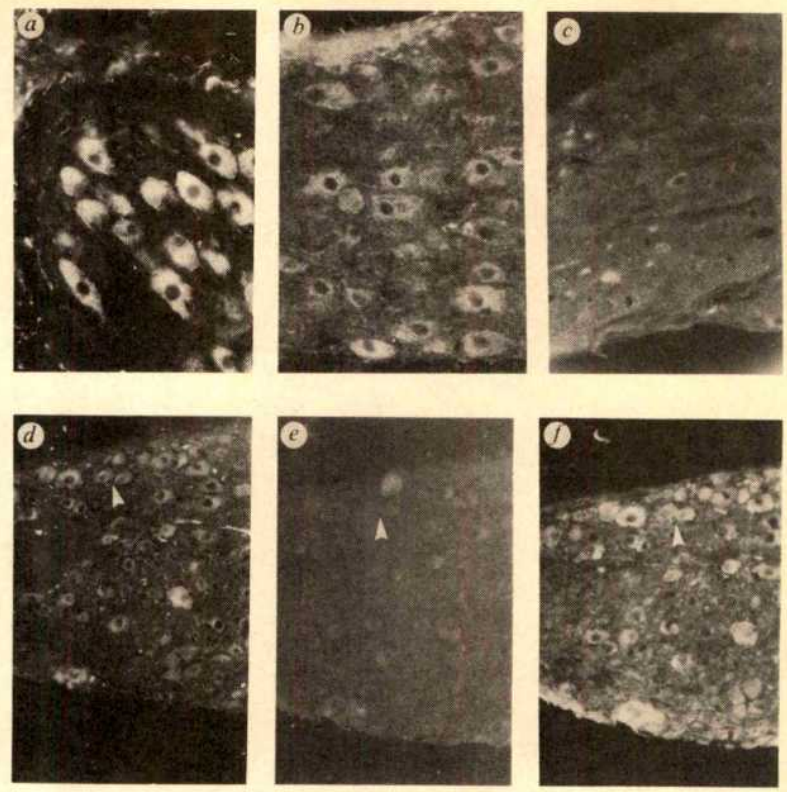


Fig. 2 Immunofluorescence micrographs of vasopressin-like peptide in sympathetic ganglia. *a*, High power view of the edge of a macaque monkey (*Macaca cynomologous*) coeliac ganglion stained for AVP (×154). Note that the nuclei are unstained. *b*, High power view of rat superior cervical ganglion stained for AVP (×154). *c*, Rat superior cervical ganglion examined in a focal plane which shows an AVP-positive nerve fibre plexus surrounding the principal neurones (×77). The principal neurones are unfocused and as a result do not appear clearly stained. *d–f*, A representative double-staining series using the elution method of Tramu *et al.*[18]. *d*, View of rat superior cervical ganglion stained for AVP (×98). *e*, View of the same section after elution of anti-AVP antibody and re-staining with second antiserum (TRITC swine anti-rabbit IgG) to confirm successful elution (×98). The exposure time for *e* was increased 10-fold to enhance the background for identification of neurones. *f*, View of the same section subsequently stained for the noradrenergic marker, dopamine-β-hydroxylase (DBH) using anti-DBH antiserum. All of the neurones stained in *d* are also stained in *f*. Arrows in each of *d–f* illustrate a representative pair of cells that stained for both VLP and DBH.

Methods: Animals were in all case transcardially perfused with a fixative consisting of 4% freshly prepared *p*-formaldehyde in phosphate-buffered saline (PBS). Tissues were removed and post-fixed for 12–24 h in the same fixative, then sunk in 30% sucrose in PBS overnight at 4 °C. Tissues were rapidly frozen and cut on a cryostat at 10 μm onto subbed slides. Slide-mounted sections were incubated with primary antibody for 60 min at 37 °C or overnight at 4 °C in 0.2 ml of PBS containing 0.3% Triton X-100 and 3% swine serum. Antisera (AVP, gift of Drs C. Pol and F. Van Leeuwen) were used at a dilution of 1:100. Sections were rinsed with several changes of PBS at room temperature for 30 min, then incubated for 60 min at 37 °C with secondary antiserum (Dako TRITC-labelled swine antiserum against rabbit IgG, diluted 1:50 in PBS/0.3% Triton X-100/3% swine serum). After rinsing in several changes of PBS, sections were examined by fluorescence optics in a Reichert-Jung Polyvar microscope. Controls included staining with secondary antibody (TRITC swine anti-rabbit IgG) alone and pretreatment of antisera with excess antigen. In the latter case, antisera were incubated in the presence of 10 μg synthetic AVP (CRB Biochemical) or 10 μg synthetic oxytocin (CRB)—both blocked the specific staining, suggesting that the VLP had antigenic characteristics of both AVP and oxytocin. One oxytocin antiserum (gift of Drs C. Pol and F. Van Leeuwen) produced identical results in immunofluorescence to those obtained with the AVP antibody.

Table 2 Production of inositol phosphates in sympathetic ganglia incubated with AVP

	^{3}H (d.p.m. per 10^5 d.p.m. in lipids)				
	GroPIns	InsP	$InsP_2$	$InsP_3$	*n*
a, Control	499 ± 92	2,209 ± 244	886 ± 142	182 ± 21	15
0.32 μM AVP for 15 s	496 ± 116	2,173 ± 281	1,325 ± 93†	458 ± 44*	15
b, Control	1,130 ± 150	6,060 ± 650	1,250 ± 140	670 ± 120	14
0.13 μM AVP for 90 min	1,680 ± 410	160,000 ± 31,000*	29,400 ± 5,850*	3,980 ± 1,220*	9
0.13 μM AVP and 1 μM V_1 antagonist for 90 min	1,150 ± 160	8,740 ± 2,600	1,490 ± 340	450 ± 110	9

a, Superior cervical ganglia from male Wistar rats (~225 g) were desheathed and incubated in 0.3 ml of Krebs–Ringer bicarbonate medium for 2.5 h in the presence of 7.5 μCi [2-^{3}H]inositol (16.9 Ci mmol^{-1}; Amersham) at 37 °C. The ganglia were then incubated in inositol-free medium for a further 1 h. Following incubation with Krebs–Ringer medium containing 10 mM LiCl for 5 min (to inhibit breakdown of inositol 1-phosphate[7]) the ganglia were stimulated with 0.32 μM VP for 15 s. Incubation was terminated with 0.1 ml of 10% (w/v) perchloric acid. The acid-soluble extract was neutralized with 1.53 M KOH (containing 75 mM HEPES to hold the *p*H of the neutralized extract at ~7.0). $KClO_4$ was precipitated at 0 °C and removed by centrifugation. After dilution to 5 ml with 5 mM K^+ tetraborate and 0.5 mM EDTA, the aqueous extract was applied to a small column of Dowex-1 × 10 (100–200 mesh, formate form) and inositol phosphates eluted with 15 ml 5 mM sodium tetraborate/60 mM ammonium formate (to elute glycerophosphoinositol, GroPIns), 20 ml 5 mM sodium tetraborate/150 mM ammonium formate (inositol 1-phosphate, InsP), 20 ml 0.1 M formic acid/0.4 M ammonium formate (inositol bisphosphate, $InsP_2$) and 0.1 M formic acid/1.0 M ammonium formate (inositol trisphosphate, $InsP_3$). Lipids were extracted from the acid-extracted ganglia using chloroform/methanol/12 M HCl (100:200:1 by vol.). The radioactivity of each phosphate ester was determined, and the values were then adjusted to a standard baseline by calculating the accumulation of radioactivity by reference to a standard incorporation of 10^5 d.p.m. into the lipids of one ganglion. The results presented are mean ± s.e.m. of the number of ganglia indicated. Statistical analysis was by an unpaired *t*-test. ***b***, Experimental details as in *a*, except that the V_1-vasopressin antagonist ([1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(*O*-methyl)tyrosine]8-arginine-vasopressin, a gift from Professor M. Manning) was added 5 min before AVP, and incubation was continued for 90 min.

* $P = 0.001$; † $P = 0.02$, difference from appropriate control values.

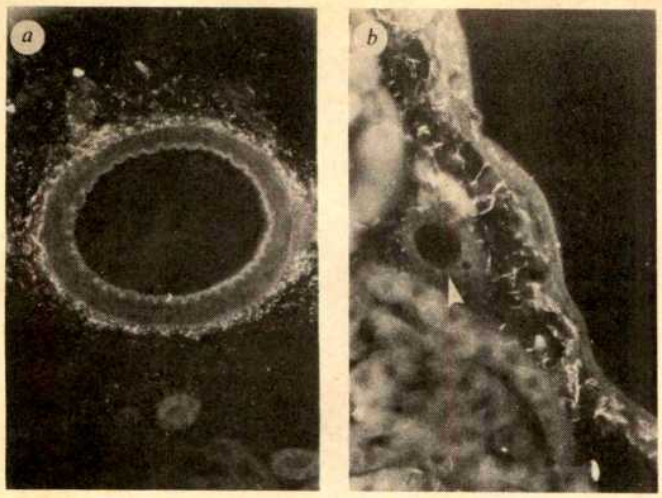


Fig. 3 Immunofluorescence micrographs of vasopressin-like peptide in peripheral nerve fibres of sympathetically innervated tissues. *a*, High power view of cross-section of mouse renal artery stained for AVP (×195). Note the dense innervation in the outer muscle layers. *b*, High power view of a hepatic blood vessel in the hilar region of rat liver (×195). Note that the innervation is highly localized to the vascular smooth muscle and is absent from the region surrounding a bile duct (arrow). Tissues were prepared and stained as described in Fig. 2 legend.

Nonetheless, using antisera to avian pancreatic polypeptide, which cross-react with neuropeptide Y[10], we have found that a smaller proportion of DBH-positive cells stain for avian pancreatic polypeptide than for VLP. In addition, the peripheral fibre distribution of immunoreactive pancreatic polypeptide is restricted to vascular beds, where it is less abundant than that of VLP-immunoreactive fibres. The only neuropeptide which has as wide and extensive a distribution of fibres in blood vessels is calcitonin gene-related peptide[11], but this is apparently of sensory nerve origin. Significantly, calcitonin gene-related peptide appears to have opposite effects to those of AVP[12], suggesting that peptidergic nerves may provide a dual control of peripheral vasculature. The antisera we used to detect VLP showed negligible reactivity with either neuropeptide Y or calcitonin gene-related peptide.

The peripheral release of VLP may explain why a variety of tissues show responses to AVP only at concentrations higher than are normally achieved by secretion into the bloodstream (see ref. 13). Furthermore, the coexistence of VLP with noradrenaline at sympathetic nerve terminals might provide a simple explanation for some non-adrenergic responses elicited by sympathetic nerve stimulation[14]. Such responses, if they are due to VLP, may be conveniently identified by inhibition with selective V_1-receptor antagonists.

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Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones

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Acidic amino acids are putative excitatory synaptic transmitters[1,2], the ionic mechanism of which is not well understood. Recent studies with selective agonists and antagonists suggest that neurones of the mammalian central nervous system possess several different receptors for acidic amino acids[3,4], which in turn are coupled to separate conductance mechanisms[5]. *N*-methyl-D-aspartic acid (NMDA) is a selective agonist for one of these receptors[3,4]. The excitatory action of amino acids acting at NMDA receptors is remarkably sensitive to the membrane potential and it has been suggested that the NMDA receptor is coupled to a voltage-sensitive conductance[6–9]. Recently, patch-clamp experiments have shown the voltage-dependent block by Mg^{2+} of current flow through ion channels activated by L-glutamate[10]. We now show using voltage-clamp experiments on mouse spinal cord neurones that the voltage-sensitivity of NMDA action is greatly reduced on the withdrawal of physiological concentrations (~1 mM) of Mg^{2+} from the extracellular fluid. This provides further evidence that Mg^{2+} blocks inward current flow through ion channels linked to NMDA receptors.

We used spinal cord neurones in dissociated cultures prepared from the ventral half or whole spinal cord of 13–14-day-old mouse embryos as described previously[11,12]. After 2–4 weeks in culture neurones were impaled with a pair of microelectrodes containing 1 M CsCl, and voltage-clamped[13]. The recording medium contained (in mM): 143 NaCl, 4.8 KCl, 5 $CaCl_2$, 10 glucose, 10 HEPES and either 0 or 1 mM $MgCl_2$ and was adjusted to *p*H 7.3; sucrose was added to match the osmolarity of the growth medium (325 mOsm). Spontaneous activity and synaptic transmission was reduced by adding 0.6 μM tetrodotoxin (TTX). NMDA (1 mM) was dissolved in bathing medium containing 0, 1 or 5 mM $MgCl_2$ and applied to neurones by pressure using pipettes with tip diameters of 1–2 μm. $CdCl_2$ (1 or 5 mM) and $MgCl_2$ (0.5–5 mM, added iso-osmotically to bathing medium) were applied to individual neurones by pressure using pipettes of tip diameter 4–8 μm. Experiments were performed at 28 °C.

Voltage recording showed that in spinal cord neurones bathed in medium containing Mg^{2+} the membrane potential depolarization evoked by NMDA was accompanied by an apparent resistance increase (Fig. 1*a*). This reflects voltage-sensitivity of the response to NMDA, as hyperpolarizing electrotonic potentials used to measure the membrane resistance result in shut off of the voltage-sensitive conductance activated by NMDA[6–9]. In contrast, when recording in Mg^{2+}-free medium, NMDA-evoked depolarizing responses were instead accompanied by a resistance decrease (Fig. 1*b*), as expected if NMDA acts to increase the membrane conductance to ions with a depolarized reversal

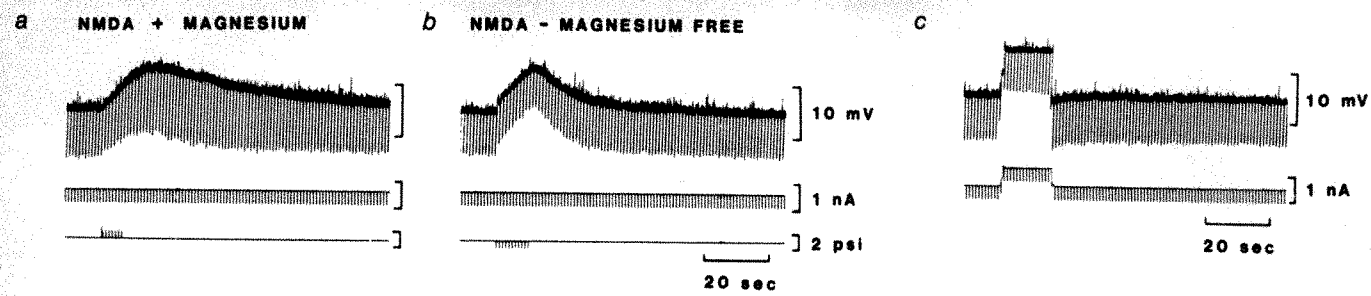


Fig. 1 The apparent membrane resistance change associated with NMDA-evoked depolarizations varies with the extracellular Mg^{2+} concentration. *a*, A depolarizing response evoked by pressure application (110 ms, 1.5 p.s.i., seven pulses) of NMDA (1mM) dissolved in medium containing 5 mM $MgCl_2$; note the apparent membrane resistance increase accompanying the NMDA-evoked depolarization. *b*, The response of the same neurone to pressure application (10 ms, 1 p.s.i., 11 pulses) of NMDA dissolved in medium to which no Mg^{2+} had been added; note the resistance decrease accompanying the NMDA-evoked depolarization. *c*, The response to a d.c. current injection, used to depolarize the membrane potential over a similar range to that occurring during the action of NMDA; note the lack of conductance change in contrast to that recorded during the action of NMDA.
Methods: Records of the membrane potential of a mouse spinal cord neurone in dissociated culture bathed in Mg^{2+}-free medium, and impaled with a pair of microelectrodes filled with 1 M CsCl. One electrode was used to record membrane potential (upper traces); the second intracellular microelectrode was used to inject hyperpolarizing rectangular current pulses (middle traces: virtual ground current monitor); these evoked electrotonic potentials used to measure membrane resistance. A pressure transducer was used to measure the timing of drug application (lower traces). The actual concentration of NMDA and Mg^{2+} at the membrane surface is unknown, but considerable dilution of the solution ejected from the pressure micropipettes is expected to occur.

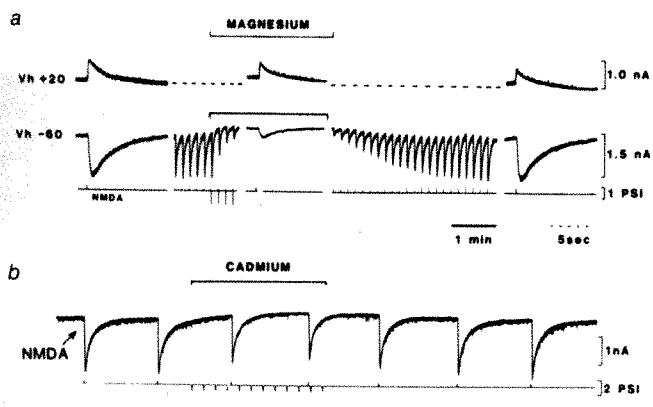


Fig. 2 Mg^{2+} antagonism of NMDA responses is selective and depends on the membrane potential. *a*, Chart records of membrane currents evoked by pressure application (30 ms, 1 p.s.i.) of NMDA (1 mM) in a spinal cord neurone voltage-clamped at holding potentials (V_h) of +20 mV (NMDA currents are outward, that is, upward) and −60 mV (NMDA currents are inward, that is, downward). Pressure application (350 ms, 1.5 p.s.i., five pulses) of Mg^{2+} (5 mM $MgCl_2$) from a second micropipette reversibly antagonized inward NMDA currents recorded at −60 mV, but had no effect on outward NMDA currents recorded at +20 mV. In Mg^{2+}-free medium NMDA currents usually showed slow inactivation, and this is the cause of the small decrease in the amplitude of the NMDA response recorded at +20 mV. *b*, Record obtained for another spinal cord neurone bathed in medium containing 10 mM tetraethylammonium chloride (to suppress outward potassium currents) showing the resistance of NMDA responses (1 mM, 1 p.s.i., 100 ms) to antagonism by doses of Cd^{2+} (1 mM, 1.5 p.s.i., 200 ms, 12 pulses) that completely blocked inward calcium currents evoked by depolarizing voltage jumps (not shown).
Methods: Spinal cord neurones were bathed in Mg^{2+}-free medium and impaled with two microelectrodes (1M CsCl, 60–100 MΩ) for voltage clamping; membrane current was measured with a virtual ground circuit (3 db, 1.6 kHz; upper traces in *a* and *b*). Pressure transducers were used to measure the period of drug application (lower trace in *a*, middle trace in *b*). In *b* the neurone was voltage-clamped at a holding potential of −50 mV, and 30-ms voltage jumps to −10 mV were used to activate voltage-sensitive inward calcium currents (5 nA peak inward current). Application of Cd^{2+} using the protocol described above blocked the inward Ca^{2+} current such that the depolarizing voltage jump then evoked 2 nA of outward leak current (similar to the inward leak current recorded by a hyperpolarizing voltage jump to −90 mV before and during Cd^{2+} application); despite this complete block of the Ca^{2+} current by Cd^{2+}, the NMDA responses recorded in this neurone (*b*) were reduced by only 15%.

potential (for example, Na^+ or Ca^{2+}). To investigate the origin of this effect of Mg^{2+} on the nature of the conductance change evoked by NMDA, it was necessary to perform voltage-clamp experiments.

NMDA evoked inward current (depolarizing) responses in all spinal cord neurones initially voltage-clamped at their resting potential. To study the antagonism by Mg^{2+} of the NMDA response, we recorded from neurones bathed in Mg^{2+}-free medium, and applied both NMDA and Mg^{2+} to individual neurones (see Fig. 2*a*). The antagonism of NMDA responses by Mg^{2+} depended closely on the membrane potential: it was strong at −60 mV, but virtually absent at +20 mV. This antagonistic action of Mg^{2+} showed noncompetitive kinetics, that is, in the presence of Mg^{2+} responses evoked by large doses of NMDA were antagonized to a greater extent than responses evoked by lower doses of NMDA. Such results could be explained if Mg^{2+} ions partially entered and blocked channels activated by NMDA, as the binding of Mg^{2+} would be influenced by the membrane electric field. Recent single-channel recording experiments provide clear evidence for a direct action of Mg^{2+} on ion channels activated by L-glutamate[10].

Experiments using intracellular recording from hippocampal pyramidal neurones have been interpreted to suggest that NMDA activates a voltage-sensitive calcium current[8]. However, cadmium (1 and 5 mM), a potent calcium-channel blocker[14], was much less effective as an NMDA antagonist than Mg^{2+} (see Fig. 2*b*). When 1 mM $CdCl_2$ was added to the recording medium no obvious change in the current–voltage relationship of responses evoked by NMDA was noted. However, in the same neurones for which cadmium had little effect on the response to NMDA, low concentrations of this potent calcium-channel blocker rapidly and completely blocked fast inward calcium currents evoked by depolarizing voltage jumps to −10 or 0 mV. Thus, NMDA does not open the same calcium channels as those gated by membrane potential alone. This result also indicates considerable specificity of the divalent cation-binding site linked to the NMDA-activated channel, with Cd^{2+} having less affinity than Mg^{2+}.

The voltage-dependent blocking action of NMDA responses by Mg^{2+} is sufficient to explain the negative slope of the NMDA current–voltage relationship recorded in media containing physiological concentrations of Mg^{2+}. Figure 3*a* illustrates the voltage dependence of the NMDA-evoked current recorded in medium containing 1 mM $MgCl_2$. The current–voltage relationship of the NMDA response has a negative slope over the potential range −70 to −35 mV, indicating that the conductance activated by NMDA becomes smaller on membrane potential

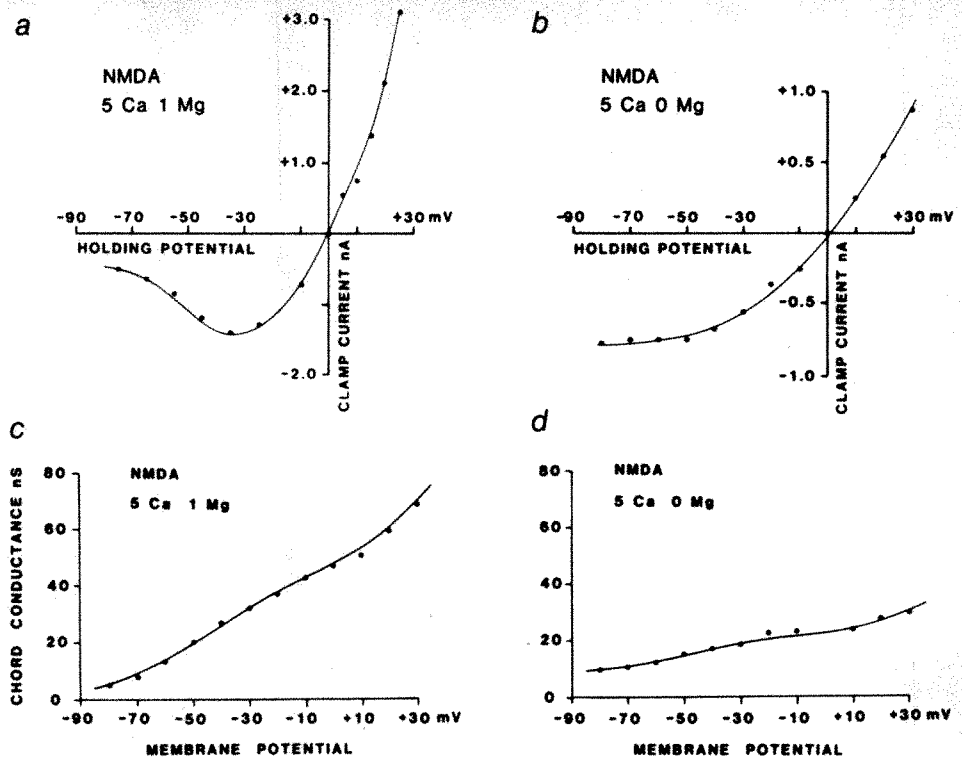


Fig. 3 Voltage-dependence of NMDA responses recorded in spinal cord neurones with and without addition of Mg^{2+} to the extracellular medium. *a*, *b*, Current–voltage plots of the peak amplitude of NMDA current responses, evoked by pressure application of amino acid to voltage-clamped spinal cord neurones, plotted against the holding potential. Each point is the mean of two responses. *a*, Obtained from a neurone bathed in medium containing 1 mM Mg^{2+}; note the negative slope of the graph between −35 and −75 mV which indicates voltage sensitivity of the NMDA response *b*, Obtained from a neurone bathed in medium to which no Mg^{2+} was added; note that the slope is positive at all membrane potentials. The residual voltage sensitivity of the NMDA response recorded in Mg^{2+}-free medium may arise from contamination of the recording medium with trace amounts of Mg^{2+} due to incomplete exchange of the Mg^{2+}-containing growth medium; additional sources of Mg^{2+} contamination may include binding of Mg^{2+} to neuronal membranes, and leakage of Mg^{2+} from the interior of cells. It is also possible that Ca^{2+} has a weak Mg^{2+}-like effect. *c*, *d*, Chord conductance–voltage plots of the NMDA responses in *a* and *b*, respectively. Note the strong outward rectification of the conductance activated by NMDA in medium containing 1 mM $MgCl_2$: the rectification ratio of the conductance at +20 mV divided by that at −70 mV is 12.2. In Mg^{2+}-free medium the NMDA-activated conductance is considerably less voltage-sensitive, and the rectification ratio recorded over the same membrane potential range is only 2.6.

Methods: Neurones were voltage-clamped as described in Fig. 2 legend and NMDA responses recorded as the membrane potential was changed by 10-mV increments. The chord (ionic) conductance membrane potential relationship was derived as follows: for a parallel conductance model the current flow through an agonist-operated channel (I_x) is related to the conductance of the agonist-operated channel (G_x) by the equation $I_x = G_x(E_m - E_x)$, where E_x is the reversal potential of the current flowing through the agonist-activated channel and E_m is the membrane potential. Thus, we could calculate the relationship between the membrane potential and the NMDA-activated conductance from our measurements of the peak amplitude of the NMDA-evoked current and its reversal potential.

hyperpolarization (see Fig. 3*c*). In contrast, when $MgCl_2$ was omitted from the recording medium the current–voltage relationship of the response evoked by NMDA did not show the characteristic negative slope behaviour described above. Instead the peak inward current evoked by NMDA increased slightly as the membrane was hyperpolarized over the potential range −40 to −70 mV (see Fig. 1*b*). Thus, removal of Mg^{2+} from the recording medium greatly reduces the voltage sensitivity of the conductance activated by NMDA (see Fig. 3*d*).

Previous experiments with NMDA have been interpreted to suggest that this amino acid has a regulatory action on an undefined voltage-sensitive conductance. Initially it was thought that NMDA closed potassium channels[14,15]. Subsequent studies suggested activation by NMDA of a voltage-sensitive calcium[8] or sodium[7,9] conductance. However, our results and those of Nowak *et al.*[10] suggest that physiological concentrations of Mg^{2+} block ion channels linked to NMDA receptors. As the block is voltage-dependent it seems probable that magnesium ions enter the channel and sense the membrane electric field. Channel block by several species of either charged or uncharged molecules is known to occur in a wide variety of membrane ion channels[17–19]; the present results extend this situation to ion channels linked to excitatory amino acid receptors. The action of Mg^{2+} at physiological concentrations suggests that acute or chronic changes in the extracellular Mg^{2+} concentration could be potent modulators of neuronal excitability *in vivo*.

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Spectral sensitivity of single cones in the retina of *Macaca fascicularis*

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Colour vision depends on the wavelength-dependent absorptions of three different photolabile pigments each located in a particular type of retinal cone. The spectral absorption of primate cones has been examined by microspectrophotometry[1–7], but this method gives information only at wavelengths where the absorption is relatively strong. Here we have analysed the absorption of two of the cones over a wider range of wavelengths by recording their electrical responses to monochromatic light. The observations were made on the retina of the monkey *Macaca fascicularis*, an animal thought to have cone pigments like those of man[8]. The measured spectral sensitivities of the red-sensitive ('red') and green-sensitive ('green') cones agreed well with estimates[9] of the cone pigment absorptions derived from colour-matching experiments in humans. At long wavelengths the sensitivity of the red cones was found to decline more rapidly than that of the green. This behaviour, attributable to the cone pigment molecules themselves, explains the paradoxical hue shift[10], in which a light of very long wavelength is perceived to be identical to a light of shorter wavelength.

A glass suction electrode was used to record photocurrent from a single cone outer segment projecting from a chopped piece of retina[11,12]. The recording chamber was continuously perfused with bicarbonate-buffered Locke's solution equilibrated with 95% O_2, 5% CO_2, and the fluid in the chamber kept near 37 °C. Light stimuli were made 'monochromatic' by interference filters of half bandwidth 10 nm. Flash strength was varied by a calibrated series of neutral density filters, and the unattenuated flux density at each wavelength was determined at the end of each day. Spectral sensitivity (the reciprocal of the flash photon density required to evoke a response of constant amplitude) was determined by the method of Naka and Rushton[13]. To prevent errors resulting from progressive bleaching or movement of the cell in the electrode, the sensitivity at each wavelength was measured between runs at 500 nm and expressed relative to the average value at 500 nm. Roughly 1,000 flashes were delivered while determining the entire spectral sensitivity over a period of about 1 h. The light was applied to the outer segment transverse to its long axis. Because of the short path length for transverse incidence, self-screening of the pigment can be neglected and measured sensitivity should be proportional to the molecular extinction coefficient of the pigment in the cone.

Outward-going photocurrents up to 20 pA in saturating amplitude were elicited by brief flashes. Figure 1*a* shows a family of flash responses from a red cone and Fig. 1*b* a similar family from a green cone. Flash strengths were increased by factors of about two. The photocurrents were diphasic, with the recovery phase marked by an underswing. The time to peak of the average response to a dim flash was 60–100 ms in six of the seven cells studied, while in one unusually slow green cone it was 150 ms. The small sample of cells provided no evidence for a systematic difference between the kinetics of the responses of red and green cones. The cone responses were, however, two to three times faster than those recorded from single rods in similar preparations from this species[12]. As expected from the principle of univariance[13], the waveforms of a cone's responses to any two monochromatic lights were identical when the light intensities were adjusted to give responses of equal amplitude.

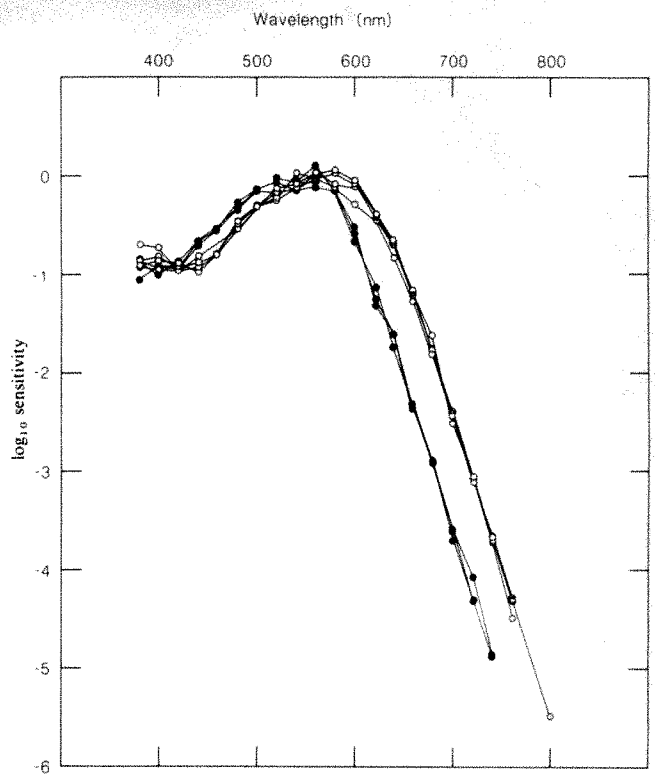


Fig. 2 Collected spectral sensitivities from seven cones. Open symbols, red cones, closed symbols, green. Lines drawn to connect each set of points. Curves positioned vertically to minimize the difference between curves in each population and to give a maximum near log $S = 0$. Sensitivity determined as described in text. Results from two male monkeys. One red cone was from the foveal region, all others from the peripheral retina.

Spectral sensitivity curves from seven cells are plotted in Fig. 2. There was no pre-selection of data; a cell was studied if it gave large responses and did not have the physiological characteristics of a rod[12]. The results fell into two groups with spectral maxima at roughly 540 nm (green cones) and 570 nm (red). Microspectrophotometry has given values near 535 and 567 nm for the average peak absorption of these two types of cones in *Macaca fascicularis*[4,5] and similar values (531, 558 nm) in human

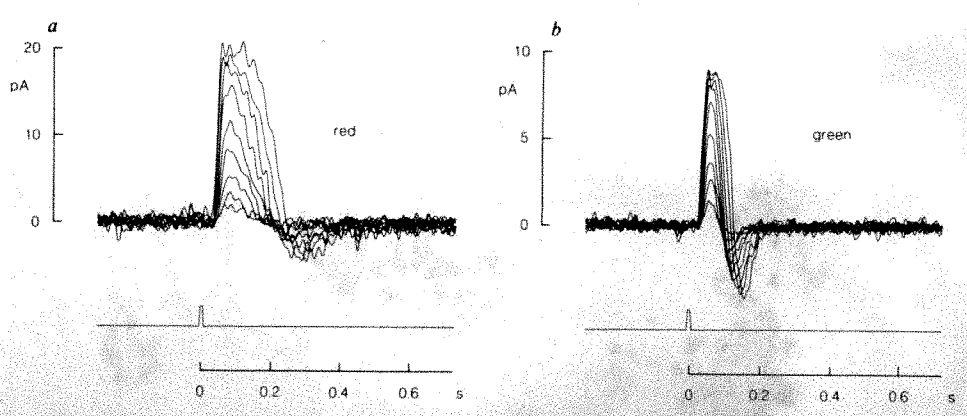


Fig. 1 Superimposed responses to brief flashes of increasing strength from a red cone (*a*) and a green cone (*b*) of the peripheral retina. Outward change in membrane current plotted as a function of time after the flash. Light monitor trace below. Each response averaged from up to 10 sweeps. Flash strengths were increased by nominal factors of 2. In *a*, flashes were at 579 nm and the intensities were raised from 292 to 40,210 photons μm^{-2}; in *b*, 559 nm and 453 to 65,640 photons μm^{-2}. Bandwidth, 0–50 Hz. Temperature, 37 °C.

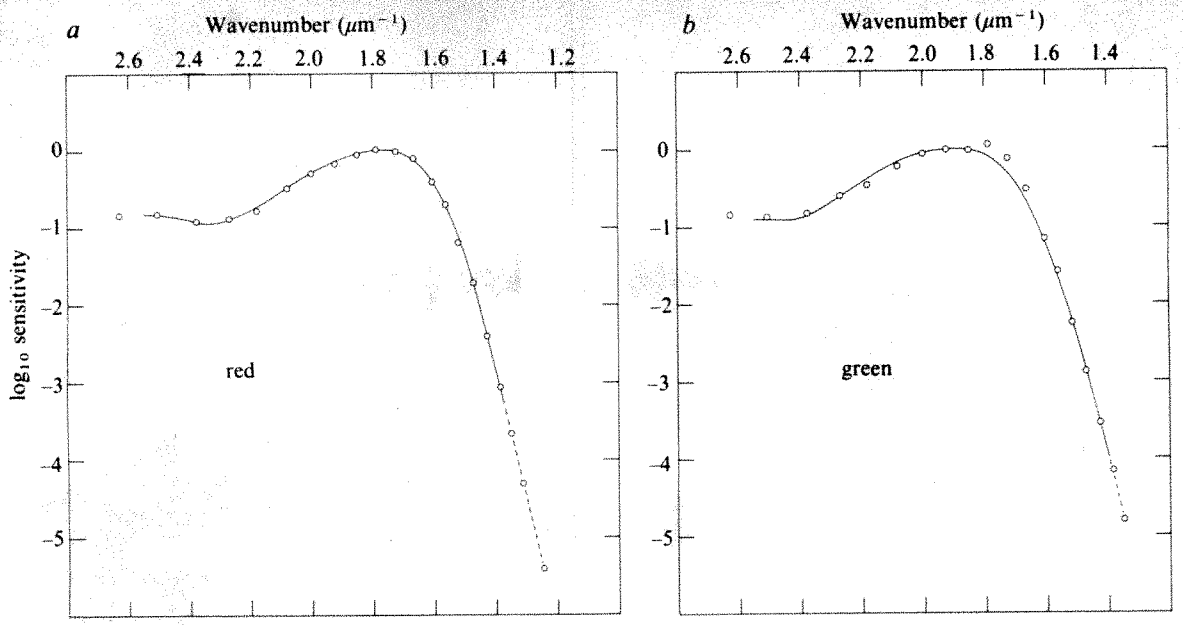


Fig. 3 Averages of $\log_{10}$ spectral sensitivities from the four red and three green cones of Fig. 2, plotted on a wavenumber scale. Solid curves drawn according to estimates by Estevez[9] of the relative spectral absorption of red and green cone pigments in humans. Each set of points positioned on the vertical scale to best fit the curves, which have maxima at 1.79 μm^{-1} (560 nm) in *a* and at 1.85 μm^{-1} (540 nm) in *b*.

cones[7]. Blue cones have not been encountered in the present work. From curves of averaged sensitivity, the spectral bandwidths at half height were estimated as $0.37 \pm 0.2\ \mu m^{-1}$ (red) and $0.41 \pm 0.2\ \mu m^{-1}$ (green); these values are comparable to those obtained by microspectrophotometry on cones of the same species[5].

The averaged spectral sensitivities of the red and green cones are plotted on a wavenumber scale in Fig. 3. The continuous curves show derived spectral absorptions in the red and green pigments as obtained by Estevez[9] from human colour-matching experiments; the dashed extensions of the curves at low wavenumber have been drawn by eye. Estimates by Smith and Pokorny[14] were similar but did not fit our measurements as well at short wavelength. The psychophysical estimates were calculated on the König assumption[15] that dichromats simply lack one of the three pigments present in normal trichromats. The agreement between the derived and measured sensitivities supports this assumption and further emphasizes the similarity of the primary colour mechanisms in the cynomolgus monkey and man.

The gradient of descent of log sensitivity at long wavelength was steeper for the red cones than for the green as predicted on theoretical grounds by Lewis[16] in 1955. Figure 4 shows this in a wavenumber plot. The lines were obtained by linear regression of $\log_{10}$ sensitivity on wavenumber, using the points at the four lowest wavenumbers. For the red cones, regression analysis gave a gradient of $17.7 \pm 0.4\ \mu m$ (slope ± standard error; correlation coefficient $r^2 = 0.995$), while for the green it was $15.5 \pm 0.6\ \mu m$ ($r^2 = 0.986$). These gradients are less than the value of 20.0 μm derived by Stiles[17] on the assumption that the ground state vibrational energies of the pigment molecules are Boltzmann-distributed. Within experimental error the gradient of decline of the red sensitivity at long wavelength is the same as the gradient of human foveal sensitivity (17.4 μm)[18,19], as expected if at threshold only red cones mediate foveal sensation in this region of the spectrum. Because the sensitivity gradient of green cones at long wavelength was less steep, the ratio of red/green sensitivities was maximum at roughly 700 nm and less on either side. This explains the psychophysical observation that a monochromatic light beyond 700 nm has the same hue as that at a characteristic wavelength below 700 nm[10,20]. Estimates of the wavelength pairs that should give the same ratio of excitation in the red and green cones were determined from

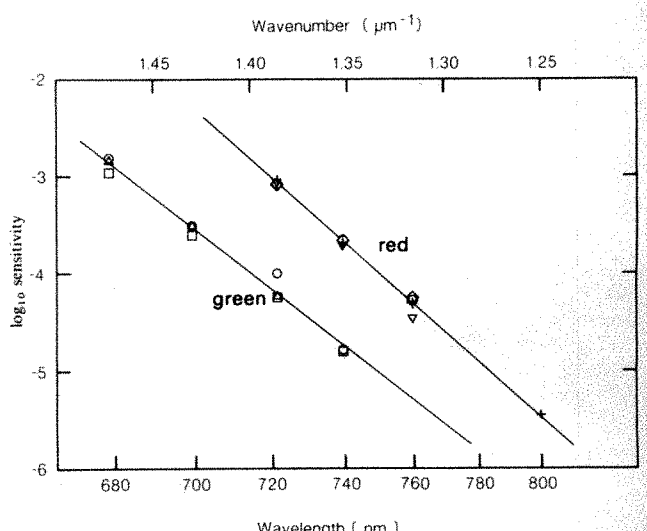


Fig. 4 Decline of sensitivity at low wavenumber. Long wavelength points from Fig. 2 replotted on a wavenumber scale, with results from each cell denoted by a different symbol. Lines obtained from the linear regression analysis described in the text.

the present results and found to be similar to the psychophysical isochromes. This suggests that the perceptual phenomenon may result in a simple way from the characteristics of absorption in the cone pigment molecules.

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Note added in proof: O. Estevez has informed us that the protanopic confusion loci on which his calculations were based are printed incorrectly in ref. 15 (p. 616). The values should read $r_{pc} = 1.0381$ and $g_{pc} = -0.0388$.

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Localized Ca^{2+} and calcium-activated potassium conductances in terminals of a barnacle photoreceptor

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Calcium channels are found in the presynaptic terminals of neurones, where they have a key role in synaptic transmission[1]. They are also found in the somata of many cells[2], in dendrites[3] and along a few axons[2,4]. In no cell is the actual distribution of these channels known in detail, because there are no known toxins or other agents suitable for labelling calcium channels, and the current through these channels is usually too small to be quantified with extracellular electrodes. However, several experiments have suggested that the density of the channels is less in the axon than in the cell body or terminal region[5–8]. Here we have used the indicator dye Arsenazo III in conjunction with an array of photodetectors to examine the spatial influx of calcium in the presynaptic terminal region of the giant barnacle, *Balanus nubilus*. In these cells, calcium entry occurs in a restricted region less than 50 μm in length, which corresponds closely to the region of synaptic contact with second-order cells. Outside this area the magnitude of calcium entry is reduced at least 50-fold. With reasonable assumptions it follows that the calcium channel density is equally localized. In addition, we demonstrate that these cells have a calcium-activated potassium conductance. Since calcium entry is restricted to the synaptic zone, this conductance must be effective only in this region.

Optical experiments using the calcium indicator dye Arsenazo III can show changes in cytoplasmic calcium concentration due to opening of calcium channels[9]. Because calcium ion diffusion within the cytoplasm is slow ($D \approx 3 \times 10^{-7}$ cm^2 s^{-1})[10] the channels must be located close to the site of calcium concentration increase. We have used an array of photodetectors[11,12] with a spatial resolution of 25 μm in the plane of the preparation to measure simultaneously the magnitude and time-course of calcium transients at many positions. Previous experiments have used a single detector and only one looked for the extent of calcium entry: Miledi and Parker[13], recording successively from two positions in the squid presynaptic terminal with a resolution of 500 μm, showed that calcium entry was greater at the synapse than at a position away from this region.

The photoreceptors of the giant barnacle are well suited for a study of calcium channel localization. From the ocellus each of the four photoreceptors sends a 10–15 mm long axon to the supraoesophageal ganglion which then bifurcates, ending in two restricted synaptic regions on the medial side of each hemiganglion (Fig. 1). The dimensions of this area are typical of many synaptic regions in both vertebrates and invertebrates and are much smaller than the squid giant synapse. The axon can be impaled along its length but the synaptic zones are too small for intracellular recording[14].



Fig. 1 Photoreceptor axon and terminal region filled with Lucifer yellow and photographed with fluorescence optics. The preparation has been fixed and cleared. The dimmer masses on each side of the terminals are the two hemi-ganglia of the supraoesophageal ganglion. The microelectrode for injecting Arsenazo III and Lucifer yellow was inserted in the axon at a point near the top of the figure. The scale bar represents 150 μm, which is also the diameter of the field sensed by the 32 elements of the photodiode array.

In normal physiological saline (461 mM NaCl, 8 mM KCl, 20 mM $CaCl_2$, 12 mM $MgCl_2$, 5 mM HEPES, *p*H 7.6) the receptor potential is conducted decrementally to the synapse. In saline containing ≥ 5 mM tetraethylammonium (TEA), a depolarizing potential will generate a calcium-dependent action potential followed by an after-hyperpolarization lasting several seconds[7]. In experiments with two microelectrodes in the axon, the amplitude of both the action potential and the undershoot were found to be larger when recorded from the electrode closer to the terminal, suggesting that the calcium channels might be more concentrated in the terminal region[7]. Pharmacological experiments gave further evidence that there were more channels in the terminal but did not improve the spatial resolution[8].

For our experiments the photoreceptor axon was impaled near the bifurcation with an electrode containing 75 mM Arsenazo III (Sigma, 8891). The dye was iontophoresed into the axon and allowed to diffuse for 15 min into the fine presynaptic terminals in each hemi-ganglion. The terminal region was imaged onto a square array of 32 photodiodes. In response to a depolarization produced by a brief light flash or electrically via the microelectrode, an absorbance increase at 660 nm was detected by photodiodes positioned over each terminal. The absorbance changes had a biphasic action spectrum consistent with an increase in ionized calcium interacting with the dye[15]. In other experiments the absorbance changes were completely eliminated when the 20 mM Ca^{2+} in the saline was replaced with 2 mM Ca^{2+} + 18 mM Co^{2+} or when 30 mM $CoCl_2$ was added to the saline. The same solutions also block the calcium-dependent action potential which can be elicited when TEA is added to the saline. These observations suggest that the absorbance changes are caused by calcium entering through membrane channels which have a pharmacology typical of other voltage-sensitive calcium channels[16].

Figure 2 shows the change in absorbance at 660 nm detected by each of 32 elements of the photodiode array. The signals have been placed over the outline of a fluorescence photograph of the terminal which was filled with Lucifer yellow after the Arsenazo experiment was completed. The largest signals are located over the final 50 μm of the terminal and are resolved with a signal-to-noise ratio of greater than 50:1. The magnitude declined by a factor of 10 within 50 μm of the terminal and no signal was detected above noise level when the array was moved to cover the axon region at the point of bifurcation in the

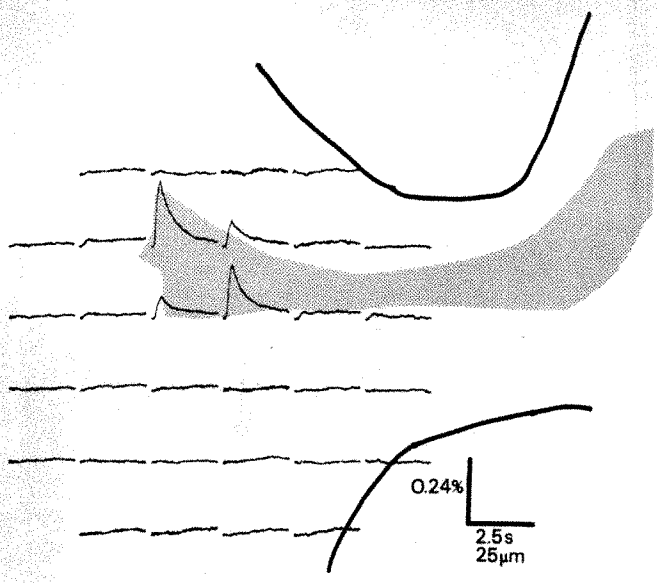


Fig. 2 Spatial distribution of Arsenazo III absorbance changes in the photoreceptor presynaptic terminal region. The stippled area traces the outline of the presynaptic terminal determined from a photograph of the live Lucifer yellow-filled photoreceptor. The heavy lines are the boundary of the ganglion. Each trace shows the stimulus-induced change in light intensity detected by a photodiode element. Fifty sweeps were averaged. The change in absorbance ΔA is proportional to $\Delta I/I$ as long as the dye does not absorb a significant fraction of the incident light, as was true for these experiments. The photoreceptor was stimulated with 250-ms depolarizing pulses through the same microelectrode used to iontophorese dye into the cell. Note that the absorbance increases during the stimulus pulse and declines to baseline with a time constant of about 1.5 s, more than five times faster than that measured at the presynaptic terminal of the squid giant synapse[22]. **Methods:** To make these measurements, the ganglion was imaged onto the photodiode array using a ×40 water-immersion lens (n.a. = 0.75). The incident wavelength was selected with interference filters having a bandwidth of 30 nm. The current output of each photodiode was converted to voltage and the d.c. component of this signal was subtracted using a sample-and-hold circuit. This signal was RC filtered with a time constant of 10 ms, amplified (×2,000), multiplexed and digitized into an LSI-11/23 computer for further analysis and display.

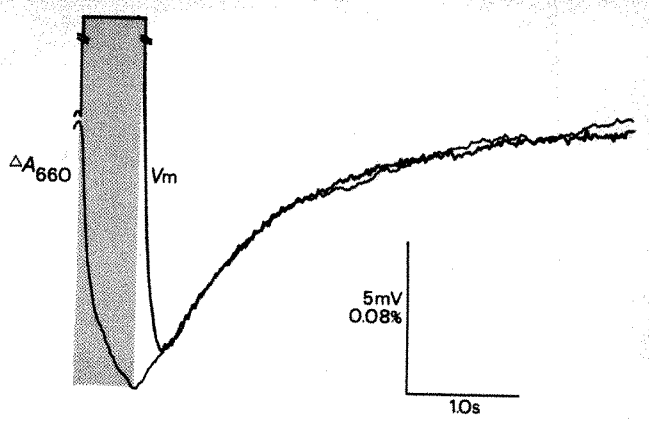


Fig. 3 Comparison of the time course of the undershoot following a depolarizing pulse (stippled area) with the simultaneously measured change in absorbance of Arsenazo III. The signal from one of the photodiodes located over the terminal region has been inverted and scaled to make the two curves coincide at the time when the potential undershoot is largest. The peak of the calcium signal coincides with the end of the depolarizing pulse. However, the peak of the undershoot is not detected at the electrode until about 100 ms later because of the time and space constants of the cell. The magnitude of the depolarizing pulse was not determined; bridge stimulation was used. Ten sweeps were averaged.

commissure. The smaller signals around the central region are probably the result of light scattering, as the signals to the top and left of the terminal come from areas where there is no terminal membrane and hence no dye. Tests with a 20-μm diameter pinhole placed under the ganglion showed that the tissue scattered the transmitted light with almost the same distribution as the absorbance signal. In addition, all of the signals have the same time course, consistent with their originating from a single locus.

In five of six experiments made using the 25-μm spacing of the grid elements, we found that the calcium signals were similarly localized to a 25–50 μm region at the end of the presynaptic process. This corresponds closely to the region of contact with the second-order cells determined anatomically[17]. In one experiment the zone of calcium entry extended over 100 μm. This may indicate that some calcium channels exist outside the synaptic zone. A more likely explanation is that the synaptic zone itself is larger than 50 μm in some preparations. The presynaptic arborization shown in Fig. 1 is an example of a cell with a larger than average arborisation. It is unlikely that the absence of signals over the region away from the terminal is due to unsusually high buffering of calcium because direct iontophoretic injection of calcium into axons previously filled with Arsenazo III gave large signals.

How does the channel density in the terminal compare with that in the rest of the axon? If we assume that the concentration of Arsenazo III is uniform in the terminal region (reasonable since the dye is injected several hundred micrometres from the terminal) and that the absorbance change is proportional to the amount of calcium entry (as shown directly by Miledi and Parker at the squid synapse[13]), it follows that the intensity change detected by each photodiode element is proportional to the amount of calcium entering the cell in the image plane of each element. Since the calcium enters through voltage-sensitive channels and the terminal is uniformly depolarized by the electrode, the signal size is proportional to the number of calcium channels. This proportionality can be extended to channel density if the amount of membrane is the same under each grid element. For this synapse, an examination of photographs of cells filled with Lucifer yellow indicates that there is probably less membrane area per grid element at the synapse than over the axon. Making the conservative assumption of equality, we conclude that the density of calcium channels in the terminal is at least 50 times higher than in the axon. This restricted distribution of calcium channels is reminiscent of the localization of acetylcholine receptor channels on the postsynaptic membrane of the neuromuscular junction[18].

The undershoot following a calcium-dependent action potential elicited in TEA saline has a reversal potential dependent on external potassium concentration and a magnitude dependent on external calcium concentration[19], suggesting that it is caused by a calcium-activated potassium conductance. Further evidence for this conclusion comes from a comparison between the Arsenazo III signal and the undershoot elicited with a depolarizing pulse in normal saline (Fig. 3). After the first 100 ms following the depolarizing pulse the potential returns to baseline with the same time course as the reduction in intracellular calcium. Because the voltage is always far from the potassium equilibrium potential, the conductance change also follows the time course of the intracellular calcium concentration change. This correspondence is expected if the average calcium concentration in the terminal parallels the concentration directly under the membrane where the conductance is controlled[20]. We have modelled the diffusion of calcium within volumes typical of the photoreceptor terminal using the methods of Zucker and Stockbridge[21]. The time to reach 90% of the equilibrium after a pulse of calcium enters the terminal was found to be $t \approx kBr^2$ where $k = 4 \times 10^{-4}$, B is the ratio of the diffusion constant of calcium in water to that in cytoplasm, and r is the radius of the synaptic terminal assumed to be a cylinder. If $B = 20$ (ref. 10)

$r = 4$ μm (ref. 17), then $t = 128$ ms, which is fast compared with the observed time course of calcium removal.

Since calcium entry is restricted to the terminal, the calcium-activated conductance change is similarly restricted. The potassium channels themselves may have a wider distribution, but those away from the terminal are not activated.

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Light-dependent calcium release from photoreceptors measured by laser micro-mass analysis

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Yoshikami and Hagins[1] first suggested that calcium is sequestered within membranous disks in the outer segments of vertebrate rods and that the bleaching of visual pigment molecules by light causes the release of Ca from the disks. Once released, the Ca was postulated to bind to Na^+ channels or carrier molecules in the plasma membrane to produce the electrical response. This theory, termed the 'calcium hypothesis', is supported by much evidence[2–5] but remains controversial, largely because of the difficulty in measuring calcium in rods and of demonstrating light-induced release[6–14]. Here we describe direct measurements of total rod Ca using a new microprobe method, called laser micro-mass analysis, or LAMMA. Using this technique, we show that rods contain large amounts of Ca concentrated in their outer segments. Physiological levels of illumination produce a graded efflux of rod Ca content, amounting to about 10^4 ions per rhodopsin molecule bleached in dim light. As light does not change the rate of Ca influx, the total Ca content of the rod decreases. In bright light, as much as half the total Ca leaves the rod during only 1 min of illumination.

The retinas of toads (*Bufo marinus*) dark-adapted for 3 h or more were removed either in darkness (with the aid of an IR converter) or in dim red light and were placed photoreceptors upwards on a Millipore filter. In the experiments of Figs 1–3, where Ca was measured only for dark-adapted rods, the dissected retina was immediately shock-frozen and processed as described in Fig. 1 legend. In the experiments of Fig. 4, where the rods were exposed to light, we first measured the electroretinogram from the isolated retina as a control for the light sensitivity of the preparation. We then sliced each retina into eight wedge-shaped pieces. One of these was immediately processed as a control, and the others were exposed to oxygenated Ringer's in darkness and in light for specified periods of time as described in Fig. 4 legend.

Ca content was analysed using a LAMMA-500 (Leybold–Heraeus, Köln, FRG). A complete description of this instrument is given elsewhere[15]. Briefly, the LAMMA-500 uses two lasers,

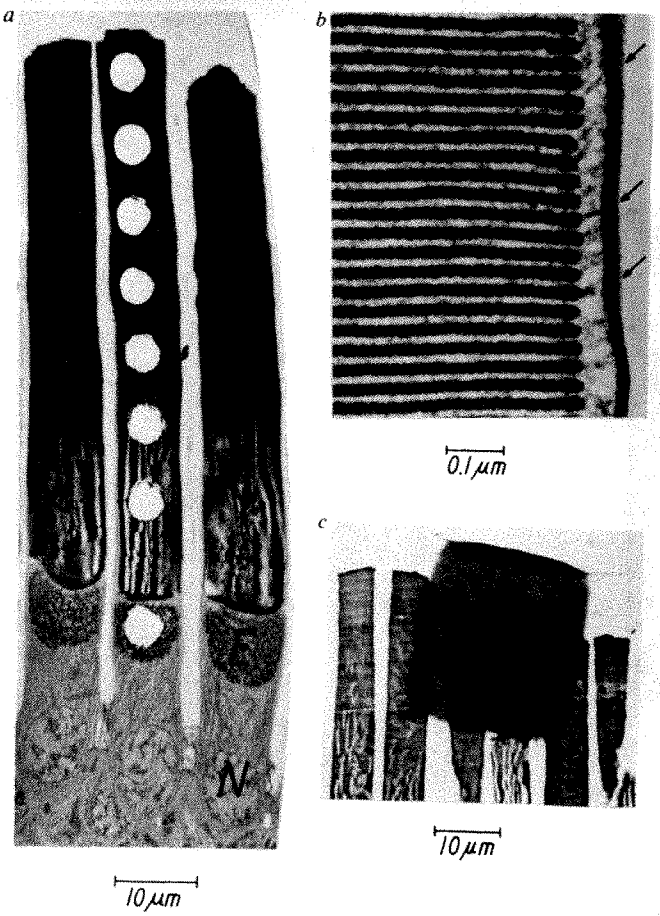


Fig. 1 Morphology and fine structure of rods prepared for LAMMA measurements. *a*, Low-power electronmicrograph of rods showing the holes produced by the high-energy laser of the LAMMA apparatus. OS, outer segment; E, ellipsoid region containing mitochondria; N, nucleus. *b*, High-power electronmicrograph taken from the distal half of the outer segment. Note the fibrillar-like densities between the disks, and the fibrillar connections between the disks and the plasma membrane (arrows; see refs 20, 21). *c*, Calibration of Ca content (see ref. 16). A 600-mesh electron microscope grid was placed directly on top of a section of retina. The section was placed, together with pellets of CaF_2, in a vacuum beam evaporator at a pressure of $<10^{-6}$ mbar. A film of CaF_2 was then deposited through the holes of the electron microscope grid onto the outer segments to produce a checkerboard-like pattern across the section. The thickness of the film was measured with a quartz crystal monitor (Balzers, QSG, 2010), which in turn was calibrated by weighing a series of standard films on a microbalance.
Methods: The tissue was prepared by rapid immersion in melting Freon at −150 °C and then in liquid N_2. In this figure and in most other experiments the frozen pieces were then transferred to vials containing dry acetone and 0.5% OsO_4 at −194 °C and were slowly warmed over the course of several days to permit the acetone gradually to replace the water in the tissue. In a few cases (for example, Fig. 2), the water in the tissue was removed by freeze-drying below −75 °C at 10^{-6}–10^{-7} mbar for 2 days. The tissue was embedded in an Epon–Araldite mixture and sectioned at a thickness of 0.5 μm on a Reichert Ultracut ultramicrotome.

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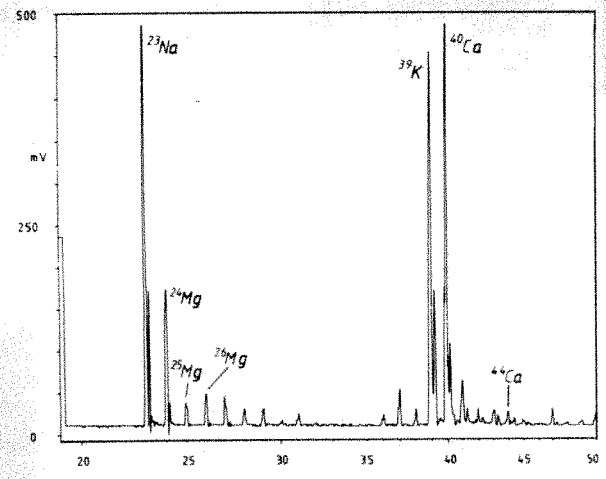


Fig. 2 LAMMA spectrum obtained for a dark-adapted rod outer segment. The spectrum was obtained from a retina shock-frozen immediately after the dissection and freeze-dried (see Fig. 1 legend). Spectra obtained from rods prepared by freeze-substitution were similar.

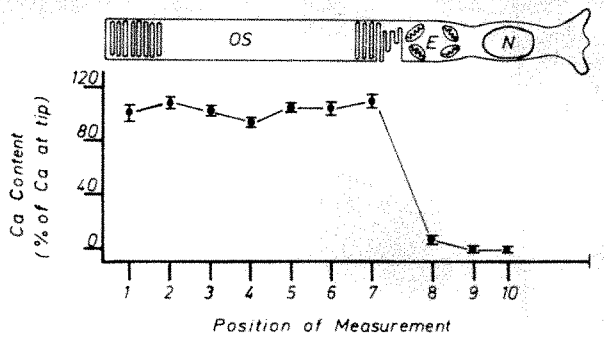


Fig. 3 Distribution of Ca content as a function of distance down the rod. For the outer segment (positions 1–7), seven equally-spaced measurements were made from each of 30 dark-adapted rods. The area within the ^{40}Ca peak at each position was then calculated on a computer after background substraction. The values for each of the 30 rods were averaged, and the means (±s.d.) were expressed as a percentage of the value at position 1. For the inner segment, separate measurements were made in each of seven dark-adapted rods for the ellipsoid (position 8), the cytoplasm between the ellipsoid and the nucleus (position 9) and for the nucleus (position 10). Means (±s.d.) of the ^{40}Ca signals are expressed as a percentage of the mean ^{40}Ca signal obtained at position 1 in the outer segment. At positions 9 and 10, the Ca was below the limit of detection of our method (50 μmol l^{-1}).

a low-power He–Ne laser and a high-power Nd:YAG pulse laser, which are colinear. The tissue sections (usually supported on an electron microscope grid) are placed in a vacuum chamber on the stage of a light microscope. The stage is moved to position the low-power laser over a region of interest, and the high-power laser is then activated to vaporize a small area 1–5 μm in diameter. Ions formed during the vaporization are accelerated into the field of an electromagnetic lens and analysed with a time-of-flight mass spectrometer.

Figure 1*a* illustrates the typical appearance of shock-frozen rods following sectioning and analysis of Ca content. The holes in the rod were produced by the high-energy laser of the LAMMA apparatus. Preservation of the tissue was best in the distal half of the outer segment, as this was directly exposed to the freezing medium (see Fig. 1*b*). In the proximal outer segment and in the inner segment there was evidence of structural damage, probably due to the formation of ice crystals. However, it was still usually possible to identify the mitochondria forming the ellipsoid region (Fig. 1*a*, E) and the nucleus (N).

Figure 2 shows the low atomic-weight region of a LAMMA spectrum from a dark-adapted rod outer segment. As the microplasma produced from the tissue by the high-energy laser contains almost exclusively singly-charged particles[15], the m/e ratios in the spectrum give the atomic weights of the analysed mass directly. The peaks for ^{23}Na, ^{39}K and the various isotopes of Mg can be clearly identified, although we cannot, with the procedures presently used, quantify the concentration of these elements in the rods. There is also a prominent peak for ^{40}Ca and a smaller peak for the less common ^{44}Ca (2% natural abundancy).

To relate the amplitude of the ^{40}Ca signal to the actual concentration of Ca within the rod, we vacuum-deposited a thin film of CaF_2 directly onto the tissue section[16], as described in Fig. 1 legend. The ratio of the LAMMA signals from rods covered by the thin film and from rods not covered by the film, measured from cells in the same section, was used to determine the total number of Ca atoms within the sampled area. To calculate the calcium concentration in the rod, we also needed to know the thickness of the section. This was determined by vacuum-evaporating platinum on both sides of the section. The section was then re-embedded, resectioned at an oblique angle, and viewed in the electron microscope. The calibration procedure was performed independently on sections of retinae from two dark-adapted animals, giving 4.4±1.8 mmol Ca per l of tissue volume (mean±s.d.) in one case and 4.7±1.8 mmol l^{-1} in the other. This represents approximately $3–5\times10^9$ Ca atoms per rod outer segment or 1–2 Ca atoms per rhodopsin[17].

As the LAMMA can produce holes that are only a few μm in diameter (see Fig. 1*a*), we used this technique to investigate differences in Ca content between different regions of the rod. These measurements (Fig. 3) show that most of the Ca in the rod (>95%) is concentrated within the outer segment. We could detect no significant variation in Ca content along the outer segment, despite large differences in the preservation of the tissue. In the inner segment, Ca could be detected only in the mitochondrial-rich ellipsoid region (250–500 μmol l^{-1}). The decrease in Ca in the inner segment could conceivably have been caused by ice crystal damage in this region. However, we could see no obvious difference in preservation between the innermost outer segment (position 7 in Fig. 3) and the outermost inner segment (position 8), even though the Ca content changed dramatically here in every rod we examined.

Figure 4 shows the effect of light on the Ca content of the rod outer segment. To distinguish efflux from influx in these experiments, we bathed the retinae in Ringer's in which the normal $^{40}CaCl_2$ was replaced by $^{44}CaCl_2$. In darkness, the ^{40}Ca concentration (●) of the rod outer segment slowly decreased and the ^{44}Ca content (◆) slowly increased. These results suggest that, in the dark, ^{44}Ca in the Ringer's slowly exchanges with ^{40}Ca inside the rod. As, however, the decrease in ^{40}Ca appears not to be entirely compensated by the increase in ^{44}Ca, some Ca may also be lost from the rods, perhaps through a gradual deterioration of our isolated retina preparation.

In continuous dim light (68 rhodopsins bleached (Rh*) per rod per s), the rate of decrease of ^{40}Ca was about three times greater than in the dark. After subtracting the 'dark efflux', the light-induced efflux at this intensity was calculated to be $\sim8.1\times10^5$ Ca atoms per rod per s, or $\sim10^4$ Ca atoms per Rh*; this is in close agreement with the value of the light-induced increase in extracellular Ca measured in toad retina using calcium electrodes[18]. As the light intensity was increased, the rate of ^{40}Ca efflux increased. At the brightest intensity we used (10^6 Rh* per rod per s), the efflux was at least 3×10^7 Ca atoms per rod per s. This is 100 times larger than the efflux in darkness.

We could detect no effect of light on the rate of Ca influx. The rate of ^{44}Ca uptake in steady illumination (Fig. 4, ◇) was indistinguishable from that in darkness within the error of the measurements. The change in total Ca concentration in the light can therefore be obtained directly from the data in Fig. 4 by adding the curve for the ^{44}Ca increase (◇) to that for the ^{40}Ca decrease at each of the light intensities. These calculations show that light causes a decrease in the total Ca concentration. In

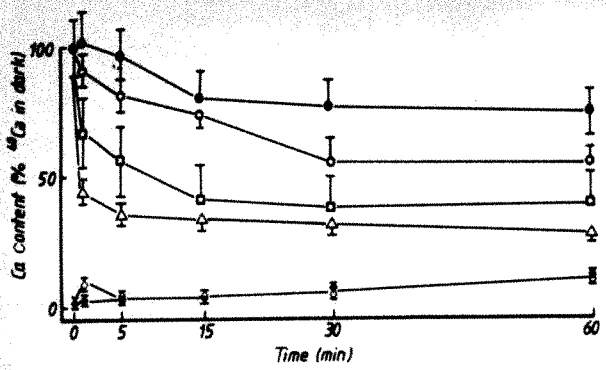


Fig. 4 Effect of light on the Ca content of the rod outer segment. The bars represent 1 s.d. Each symbol represents a different isotope or experimental condition: ●, ^{40}Ca–dark; ◆, ^{44}Ca–dark. The light intensities used for ^{40}Ca were: 68 Rh* per rod per s ○, 8.1×10^3 Rh* per rod per s □ and 10^6 Rh* per rod per s △; and for ^{44}Ca: 10^6 Rh* per rod per s ◇. Illumination was continuous.
Methods: The retina, attached to a Millipore filter, was cut into eight wedge-shaped pieces. One piece was processed immediately as a control. The other pieces were fixed to plates of dental wax with insect pins and placed in 35-mm Petri dishes containing oxygenated Ringer's of the following composition (in mmol l^{-1}): 106 NaCl, 2.5 KCl, 0.13 $NaHCO_3$, 1.5 $MgCl_2$, 1.8 $^{44}CaCl_2$, 5.6 glucose, and 3.0 HEPES at *p*H 7.8. $^{44}CaCl_2$ was taken from a stock solution prepared by dissolving $^{44}CaCO_2$ (99% pure; Rohstoffeinfuhr, Dusseldorf, FRG) in HCl. The data point at time zero is a control value determined from all retinas used in this figure, expressed as 100%. Other data points give the mean Ca content expressed as a percentage of the control at 1, 5, 15, 30 and 60 min, in each of the experimental conditions. The mean Ca content was calculated from 40 outer segments: 10 measurements were made for each of four pieces of tissue, each from a different retina. The light stimulus was a continuous 503-nm field ~50 nm in diameter. Intensity within the field was uniform within ±10%. Absolute values of intensities were measured and numbers of rhodopsins bleached calculated as described in ref. 22.

bright light, one-half to two-thirds of the total Ca in the outer segment disappeared within a few minutes. The remaining one-third to one-half appeared to be unaffected by illumination for as long as our measurements were continued.

The results presented here show that rods in the intact retina contain large amounts of Ca within their outer segments. In the dark, Ca in the rod exchanges slowly with Ca in the external medium. As the permeability of the plasma membrane to Ca seems not to be limiting (W.H.S. and G.L.F., in preparation), the slow rate of exchange may indicate that Ca is sequestered within an intracellular compartment, perhaps the disks. Exposure to light causes as much as half of this Ca to be released, most to be transported out of the rod apparently by Na–Ca exchange[18,19]. However, as much as 5% of the Ca released from the outer segment accumulates within the mitochondria of the inner segment[19], suggesting that light increases the free Ca concentration within the rod. Our results are therefore consistent with the principal postulates of the Ca hypothesis[1]. In addition, our results show that once Ca has left the rod, it is not replaced as long as illumination continues. It will be of interest to examine how the Ca content is restored once the light is extinguished, and to investigate the relationship, if any, between the re-uptake of Ca and the return of photoreceptor sensitivity during dark adaptation.

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ATP-stimulated interaction between epidermal growth factor receptor and supercoiled DNA

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The receptor for epidermal growth factor (EGF) has been identified as a transmembrane glycoprotein that has tyrosine-specific kinase activity[1]. The kinase activity of the receptor is enhanced in the presence of EGF (or related peptides), and the phosphorylation of a number of substrates, as well as autophosphorylation of the receptor, has been reported[1]. Analogous findings have been described for the insulin receptor and the receptor for platelet-derived growth factor (PDGF)[2]. Thus, a number of hormone receptors and several viral transforming proteins[3] appear to share the highly unusual property of tyrosine-specific kinase activity. Nevertheless, the specific relationship between tyrosine kinase activity and the control of cell growth and replication is unknown. It is known that after the initial binding of EGF to the plasma membrane, the hormone together with its receptor is rapidly internalized in endocytic vesicles and the hormone is eventually degraded in lysosomes[4]. It is possible that the function of EGF is simply to stimulate internalization of its receptor, and that as a result of its altered location the receptor is able to phosphorylate a cytoplasmic component or even interact directly with a nuclear component. We now report that the purified receptor for EGF is able to interact with and nick supercoiled double-stranded DNA in an ATP-stimulated manner.

The receptor for EGF was isolated from membrane vesicles of A-431 cells[5]. The final purification steps involved EGF-Affi Gel chromatography, wheat germ lectin–Sepharose chromatography and DNA cellulose chromatography. Figure 1*c* displays a silver stained 10% SDS-polyacrylamide gel of purified 150,000–170,000 molecular weight (MW) (lane 1) and 150,000 MW (lane 2) receptor preparations isolated from A-431 cells. Incubation of the EGF receptor with supercoiled pBR322 plasmid DNA converts form I DNA (supercoiled) to form II DNA. This conversion is ATP-dependent. Assays of equivalent aliquots from each step in this purification are shown in Fig. 1*a*. Both the kinase activity and the DNA nicking activity co-purify during the EGF-Affi Gel, wheat germ lectin–Sepharose and DNA cellulose purification steps. The purified EGF receptor also reacts with partially relaxed topoisomers of pBR322

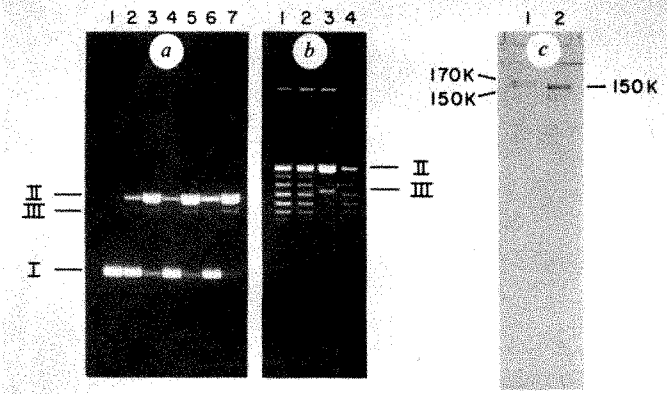


Fig. 1 Agarose gel electrophoresis of pBR322 plasmid DNA after incubation with the EGF receptor in the presence or absence of ATP. *a*, Interaction of supercoiled pBR322 DNA (form I) with various preparations of the EGF receptor. Lane 1, 0.8 μg of form I DNA; lanes 2 and 3, 0.8 μg of form I DNA incubated with wheat germ lectin purified EGF receptor in the absence (lane 2) or presence (lane 3) of 5 mM ATP; lanes 4 and 5, 0.8 μg of form I DNA incubated with wheat germ lectin purified EGF receptor passed through single-stranded DNA agarose in the absence (lane 4) or presence (lane 5) of 5 mM ATP; lanes 6 and 7, 0.8 μg of form I DNA incubated with wheat germ lectin purified EGF receptor passed through double-stranded DNA cellulose in the absence (lane 6) or presence (lane 7) of 5 mM ATP. *b*, Interaction of partially relaxed preparations of pBR322 (topoisomerase I-treated pBR322 DNA) with the EGF receptor. Lane 1, 0.8 μg of enzymatically relaxed pBR322 DNA; lanes 2–4, 0.8 μg of relaxed pBR322 DNA incubated with the EGF receptor in the absence of ATP (lane 2), the presence of 5 mM ATP (lane 3), or the presence of 5 mM AMP (lane 4). *c*, Silver stained 10% SDS-polyacrylamide gel of the 170,000/150,000 MW (lane 1) and 150,000 MW (lane 2) EGF receptor preparations purified by EGF Affi-Gel, wheat germ lectin–Sepharose and DNA-cellulose chromatography.

Methods: Agarose gel electrophoresis was performed in 40 mM Tris-acetate, *p*H 7.9, 20 mM sodium acetate at room temperature for 12 h at 3 V cm^{-1}. The 0.8% agarose gel (*a*) and 1.0% agarose gel (*b*) were stained with ethidium bromide and photographed with UV illumination. The preparation of A-431 membrane vesicles and subsequent EGF Affi-Gel purification of the EGF receptor has been described elsewhere[5]. The EGF Affi-Gel purified receptor was adsorbed to a wheat germ lectin–Sepharose column and the receptor was eluted with 3 mM *N*, *N'*, *N''*,-triacetylchitotriose essentially as described for the mouse liver EGF receptor[6]. DNA-cellulose (P-L Biochemicals) chromatography and single-stranded DNA agarose (BRL) chromatography of the wheat germ lectin purified receptor were performed in buffer comprising 0.2% Triton, 10% glycerol, 20 mM HEPES, *p*H 7.4 and 2 mM $MgCl_2$. Approximately one bed volume of wheat germ lectin purified receptor was added to the DNA matrix and adsorption was allowed to take place 30 min on ice with stirring. The receptor preparation did not bind to either matrix. (These DNA columns were used in an attempt to remove any trace nuclease contamination.) The conversion of supercoiled (form I DNA) to nicked DNA (form II DNA) was carried out in 20 mM HEPES, *p*H 7.4, 5 mM $MgCl_2$ and 5 mM ATP in a reaction volume of 30 μl. The reaction was terminated either by the addition of SDS (2%) or by phenol extraction followed by ethanol precipitation. The amount of purified receptor added per reaction mixture varied from 0.2 μg to 0.8 μg. The conversion of form I DNA to form II DNA, however, can be detected with as little as 0.02 μg of purified receptor. In all the experiments described here, incubation was allowed to proceed for 25 min at room temperature and unless specified otherwise the receptor preparation was purified by both wheat germ lectin–Sepharose chromatography and single-stranded DNA agarose chromatography. pBR322 plasmid DNA (derived from *Escherichia coli* strain NH 7007) was purified by Sephacryl S-1000 chromatography followed by caesium chloride buoyant density centrifugation[8]. Wheat germ topoisomerase I (Promega Biotec) was used to partially relax supercoiled pBR322 DNA.

(topoisomerase I-treated preparations of pBR322 DNA) in the presence of ATP to yield form II DNA (Fig. 1*b*). This conversion does not occur when AMP is substituted for ATP (Fig. 1*b*, lane 4).

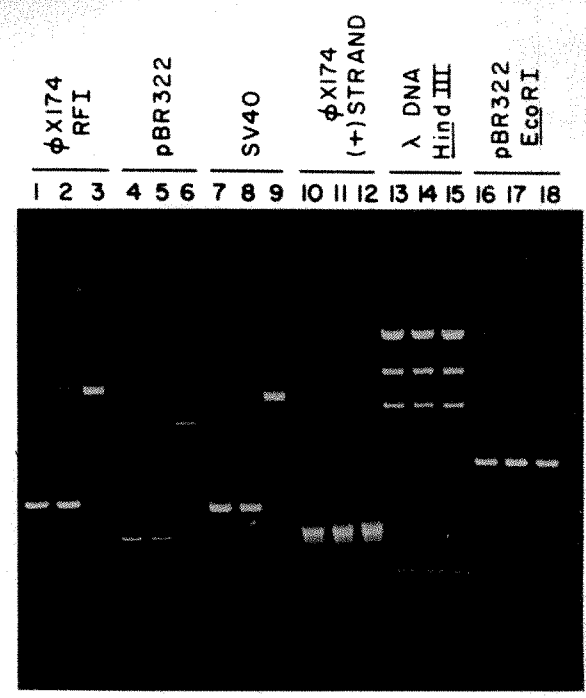


Fig. 2 Specificity of the EGF receptor–DNA interaction. Purified EGF receptor was incubated with different DNA templates in the presence or absence of ATP and the DNA products were analysed by agarose gel electrophoresis. Lanes 1–3, ΦX174 RFI DNA (0.75 μg) was incubated in the absence of the EGF receptor (lane 1), or in the presence of EGF receptor without (lane 2) or with (lane 3) 5 mM ATP; lanes 4–6, plasmid pBR322 DNA (0.3 μg) incubated in the absence of the EGF receptor (lane 4), or in the presence of EGF receptor without (lane 5) or with (lane 6) ATP; lanes 7–9, supercoiled SV40 DNA (0.9 μg) incubated in the absence of receptor (lane 7) or in the presence of receptor without (lane 8) or with (lane 9) ATP; lanes 10–12, ΦX174 single-stranded (+) DNA incubated in the absence of receptor (lane 10), or in the presence of receptor without (lane 11) or with (lane 12) ATP; lanes 13–15, a *Hin*dIII digest of DNA (1.8 μg) incubated in the absence of receptor (lane 13) or in the presence of receptor without (lane 14) or with (lane 15) ATP; lanes 16–18, *Eco*RI digest of pBR322 (0.6 μg) incubated in the absence of receptor (lane 16) or in the presence of receptor without (lane 17) or with (lane 18) ATP. Where indicated, the DNA was digested to completion by the appropriate restriction enzyme (New England Biolabs), phenol extracted and ethanol precipitated prior to addition to the reaction mixture. The DNA products were fractionated on a 1% agarose gel.

The ATP-dependent conversion of supercoiled (form I) pBR322 to form II is observed when either the 170,000/150,000 MW receptor preparations or the 150,000 MW proteolytic cleavage product of the native 170,000 MW A-431 EGF receptor is utilized in the reaction mixture. Both the 170,000 MW and the 150,000 MW forms of the receptor have been shown to possess binding activity for EGF and EGF-stimulated tyrosine-specific kinase activity[5]. The 170,000 MW EGF receptor isolated from normal mouse liver[6] also possesses the ATP-dependent nicking activity for form I pBR322 DNA (data not shown). It is of interest that in these assay conditions the activity is not significantly altered by the addition of EGF to the reaction mixture; in contrast, EGF enhances the tyrosine kinase activity of the receptor[5].

The specificity of the interaction of the EGF receptor with DNA was examined by incubating the EGF receptor, in the presence or absence of ATP, with a variety of nucleic acid substrates and examining the reaction products by agarose gel electrophoresis.

As illustrated in Fig. 2, the conversion of form I plasmid DNA to form II DNA is not specific for pBR322; ΦX174 RFI DNA (Fig. 2, lanes 1–3), simian virus 40 (SV40) DNA (Fig. 2, lanes 7–9), and PM2 DNA (data not shown) react in a fashion similar to pBR322 DNA (Fig. 2, lanes 4–6). Regardless of the

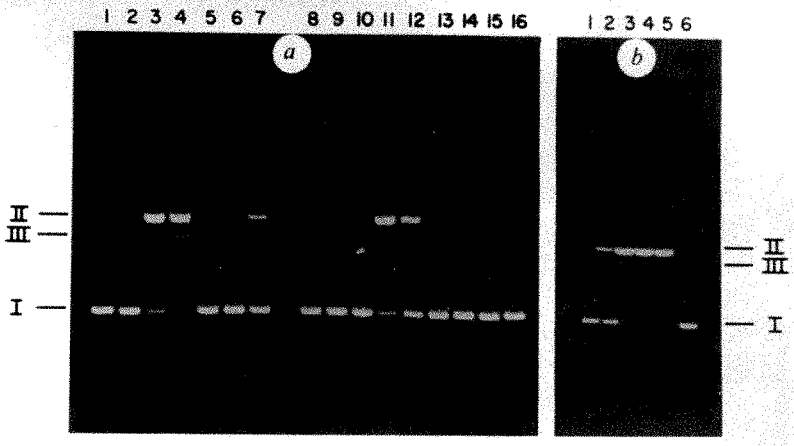


Fig. 3 Nucleotide preference and heat stability of the EGF receptor-associated DNA-relaxing activity. ***a***, Plasmid pBR322 was incubated with the EGF receptor in standard assay conditions (see Fig. 1) in the presence of various nucleotides. Lanes 1 and 8, pBR322 DNA (0.8 μg) incubated without EGF receptor; lanes 2–16, pBR322 DNA (0.8 μg) incubated with the EGF receptor in the absence of nucleotide (lanes 2 and 9) or with 5 mM ATP (lane 3), 5 mM dATP (lane 4), 5 mM 5′-AMP-PNP (lane 5), 5 mM dAMP (lane 6), 5 mM ADP (lane 7), 5 mM TTP (lane 10), 5 mM CTP (lane 11), 5 mM UTP (lane 12), 5 mM GTP (lane 13), 20 mM GTP (lane 14), 5 mM dGTP (lane 15), and 20 mM dGTP (lane 16). ***b***, Heat inactivation of the DNA-relaxing activity of the EGF receptor. Receptor preparations were heated in a water bath at the indicated temperature before addition to the reaction mixture. Lane 1, pBR322 DNA (0.8 μg) incubated in reaction mixture plus ATP but minus the EGF receptor; lane 2, pBR322 DNA incubated with the EGF receptor but minus ATP; lane 3, pBR322 DNA incubated in the presence of both ATP and the EGF receptor; lanes 4–6, pBR322 DNA incubated with ATP and the EGF receptor that had been heated for 15 min at 45 °C (lane 4), 15 min at 65 °C (lane 5), and 5 min at 90 °C (lane 6) before addition to the reaction mixture. Reaction products were fractionated by electrophoresis in 0.8% agarose and stained with ethidium bromide.

source of the closed duplex DNA a small amount of linearized product (form III DNA) is always detected in the presence of ATP and the EGF receptor. In control experiments neither linearized pBR322 (Fig. 2, lanes 16–18) nor a *Hin*dIII digest of DNA (Fig. 2, lanes 13–15) appeared to be affected by incubation with the EGF receptor in the presence of ATP. Furthermore, using nick-translated ^{32}P-labelled rabbit pox virus DNA or uniformly ^{3}H-labelled total cellular RNA from vesicular stomatitis virus (VSV) infected cells as substrates we could detect only a trace of nuclease activity (trichloroacetic acid soluble radioactivity) with the former substrate (<0.5% digestion) and none with the ^{3}H-labelled RNA after incubation for 3 h at room temperature in the presence of the EGF receptor and ATP. Thus appreciable enzymatic digestion of these nucleic acids was not detected.

Although the data suggest that the observed activity is intrinsic to or tightly associated with the receptor, we cannot unequivocally rule out the possibility that what we are observing is a low level of ATP-dependent, lectin-adsorbable endonucleolytic contamination which is observed solely in a highly sensitive assay such as the conversion of form I DNA to form II DNA, and which is present in both forms of the EGF receptor prepared from A-431 cells and the receptor prepared from mouse liver. However, no nicking activity was detected when highly purified preparations of tubulin, bovine serum albumin, IgG, EGF or PDGF were assayed.

Some of the biochemical properties associated with the DNA-nicking activity of the EGF receptor are depicted in Fig. 3. Figure 3*a* demonstrates that both UTP and CTP as well as dATP can substitute for ATP in the reaction mixture. However, the non-hydrolysable analogue of ATP, 5′-AMP-PNP, is not capable of replacing ATP, nor are GTP, dGTP, or TTP effective. The optimum ATP concentration for this reaction is 2–7 mM (data not shown). The activity is extremely heat stable (Fig. 3*b*), withstanding incubation at a temperature of 65 °C for 15 min and requiring incubation at 90 °C for at least 5 min for complete loss of activity. This thermal inactivation profile is profoundly different from that observed for the tyrosine kinase activity intrinsic to the EGF receptor. Over 80% of the tyrosine kinase activity of the EGF receptor is inactivated by heating for 15 min at 45 °C, while the EGF binding activity of the EGF receptor requires heating for 15 min at 65 °C for inactivation[7]. The observed ATP-dependent DNA reaction appears to have a *p*H optimum of 6.8–7.6 and is drastically inhibited by relatively low salt concentrations (100 mM KCl or NaCl).

To ascertain whether the reaction products are nicked or whether they represent relaxed, covalently closed circular DNA, agarose gel electrophoresis was performed in the presence or absence of ethidium bromide. In the presence of ethidium bromide the relative migration of a relaxed species of plasmid

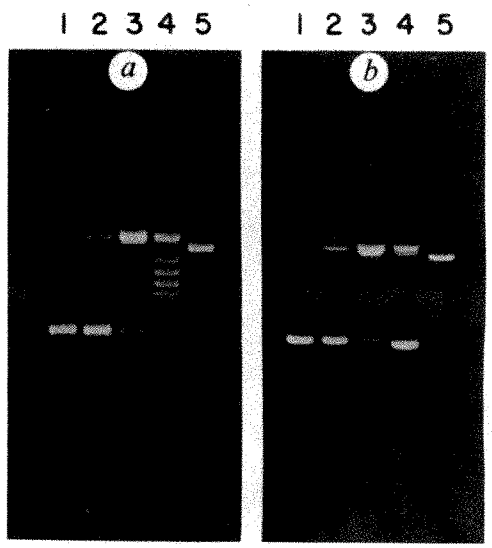


Fig. 4 Comparison of electrophoretic mobilities of pBR322 DNA reaction products in the presence or absence of ethidium bromide. ***a***, Agarose gel electrophoresis in the absence of ethidium bromide. Lanes 1–3, pBR322 DNA (0.4 μg) incubated in the absence of EGF receptor (lane 1), in the presence of EGF receptor without ATP (lane 2), or in the presence of EGF receptor plus ATP (lane 3); lane 4, pBR322 DNA (0.6 μg) enzymatically relaxed with wheat germ type I topoisomerase; lane 5, pBR322 DNA treated with *Eco*RI. 0.7% agarose gel was electrophoresed in 40 mM Tris-acetate, *p*H 7.9, 20 mM sodium acetate at room temperature for 12 h at 3 V cm^{-1}. ***b***, Agarose gel electrophoresis run exactly as in *a*, except for the inclusion of ethidium bromide (0.5 μg ml^{-1}) in both the gel matrix and running buffer. Aliquots of reaction mixtures described in *a* were brought to a final concentration of 0.5 μg ml^{-1} of ethidium bromide before electrophoresis. Lane 1, 0.4 μg of pBR322; lane 2, 0.4 μg of pBR322 incubated in the presence of EGF receptor minus ATP; lane 3, 0.4 μg of pBR322 incubated in the presence of EGF receptor and 5 mM ATP; lane 4, 0.6 μg pBR322 enzymatically relaxed with wheat germ type I topoisomerase; lane 5, *Eco*RI-treated pBR322. Electrophoresis was performed in the dark to minimize nicking of the DNA.

DNA should change as the molecule progressively binds more ethidium bromide to become increasingly positively supercoiled (compare lanes 4 in Fig. 4*a* and *b*) while that of a nicked species should not change in the presence of the intercalating dye. Comparison of lanes 3 in Fig. 4*a* and *b* indicates that the reaction product generated in our conditions in the presence of both ATP and the EGF receptor represents a nicked form of plasmid DNA since the relative mobility of form II DNA is not affected by the presence of ethidium bromide.

In view of the biological implications of the observations, and the association of tyrosine kinase activity with both the EGF receptor and pp60^{src}, we have examined three preparations of pp60^{src}. All three preparations generated reaction products apparently identical to those generated by the EGF receptor in the same conditions (data not shown). The DNA-nicking activity of pp60^{src} is also dependent on ATP.

In a number of isolated cases we have observed the generation of a high molecular weight DNA species (possibly representing concatenated DNA) exclusively in the presence of ATP and the EGF receptor. The generation of relaxed topoisomers from covalently closed supercoiled DNA in the presence of pp60^{src} has also occasionally been observed. It is possible that the enzymatic activity reported here is both a nicking and a closing activity reminiscent of other DNA interacting proteins such as DNA topoisomerases. Final proof that this activity is intrinsic to both the EGF receptor kinase and pp60^{src} is not yet available. However the association of DNA-nicking activity with tyrosine kinase preparations from diverse sources is intriguing. A nuclear translocation of these membrane-associated proteins, perhaps via endocytic vesicles and subsequent interaction with DNA could conceivably be responsible for some of the pleiotropic responses accompanying growth stimulation.

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Activation of cellular gene by mouse mammary tumour virus may occur early in mammary tumour development

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There is now good evidence that the induction of mammary carcinomas by mouse mammary tumour virus (MMTV) involves provirus activation of specific cellular genes. Thus, a high percentage of virally induced tumours contain an acquired MMTV provirus in either of two defined integration regions, termed *int-1* and *int-2*, and provirus insertion is accompanied by expression of specific RNA transcripts from these regions[1–4]. We show here that in some recurring, pregnancy-dependent mammary tumours provirus integration within *int-2* has already occurred at the earliest appearance of the tumour and may therefore represent an important step in the development of neoplasia. As judged by the distribution of the acquired MMTV proviruses, the tumours recurring at any one site represent the same clonal population of cells as the original tumour and remain clonal during cycles of proliferation and regression, including the transition to hormone-independent status. These data suggest that either the expression of the *int-2* locus or the function of this putative oncogene must remain responsive to hormones and that some additional event must be responsible for the transition to autonomous growth.

Although MMTV is one of the principal causative agents in the development of mouse mammary tumours, the incidence and progression of the disease is multifactorial, being influenced by such parameters as genetic background, parity and hormonal status of infected animals[5,6]. In BR6 mice, which suffer a mammary tumour incidence of over 90% as a result of milk-borne MMTV[7], tumours develop to a detectable size only after an average of four pregnancies and ~70% of them are initially hormone-dependent in that they regress completely or partially between pregnancies[8,9]. In subsequent pregnancies, the tumour becomes re-established at the same site but eventually progresses to become autonomous and to grow independently of the hormonal status of the animal.

Female BR6 mice were examined at each pregnancy for the presence of detectable tumours and, where possible, surgical biopsies were taken amounting to ~50% of the tumour mass. The aim was to collect repeated biopsies from the same site at each recurrence as the tumour progressed from a hormone-dependent to independent phenotype. As illustrated in Fig. 1, the number of pregnancies required to establish hormone independence was variable, ranging in the examples studied from two at tumour site B to at least six at tumour site A. Figure 1*d* includes an example of a tumour series from which no biopsies were taken, in order to illustrate the more typical pattern not distorted by the surgical removal of tissue.

DNA of high molecular weight was prepared from each fresh biopsy and analysed by Southern blotting procedures using MMTV-specific probes (see Fig. 2 legend for details). As shown in Fig. 2*b*, normal tissues in the BR6 mouse (track N) contain three endogenous proviral units of MMTV. Such endogenous sequences are present in all tissues and on both chromosomes, whereas newly acquired MMTV proviruses occur on only one of any pair of chromosomes. Thus, in a truly clonal population of infected cells, the intensity of the hybridization signal from an acquired provirus should be half that of an endogenous unit. In practice, the quantitation can be distorted by the inclusion of any normal tissue in the tumour biopsy, but the data presented here, and in many other studies[1,2,10–13], indicate that mammary tumours approach this idealized clonal situation. In all the pregnancy-dependent series examined at least one pair of novel junction fragments was present in DNA from the first biopsy, indicating that acquisition of additional MMTV sequences is probably an early event in tumour induction. Moreover, identically sized, novel fragments were detectable in all subsequent biopsies from the same site, suggesting that recurrence of the tumour at each pregnancy results from expansion of the same, clonal population of tumorigenic cells.

Although the initial pattern of acquired MMTV proviruses remained stable during subsequent cycles of proliferation and regression, DNA from the later biopsies occasionally showed a more complicated pattern (for example tumour sites B and C in Fig. 2). Since the changes were invariably additive, and were not accompanied by loss or rearrangement of established proviruses, they presumably resulted from superinfection of the tumour tissue by MMTV. Judging from the intensities of these additional junction fragments on the autoradiograph, superinfection must have occurred either before or very early in a period of proliferation such that a high percentage of tumour cells contained the same fragments. Since most of the cells in a tumour nodule are presumably lost during regression, each recurrence of the nodule must originate from perhaps a single or, at most, a small number of tumorigenic cells and will therefore only reflect the proviral pattern in this or these cells. It is possible that these represent a stem cell population whose capacity to differentiate in response to the hormonal influences accompanying pregnancy remains unimpaired after infection and possibly 'transformation' by MMTV.

At this stage, we cannot formally exclude the possibility that the acquisition of additional MMTV sequences may have contributed to the transition of tumours to autonomous growth. However, since examples have been found in which hormone independence was apparently achieved without further provirus

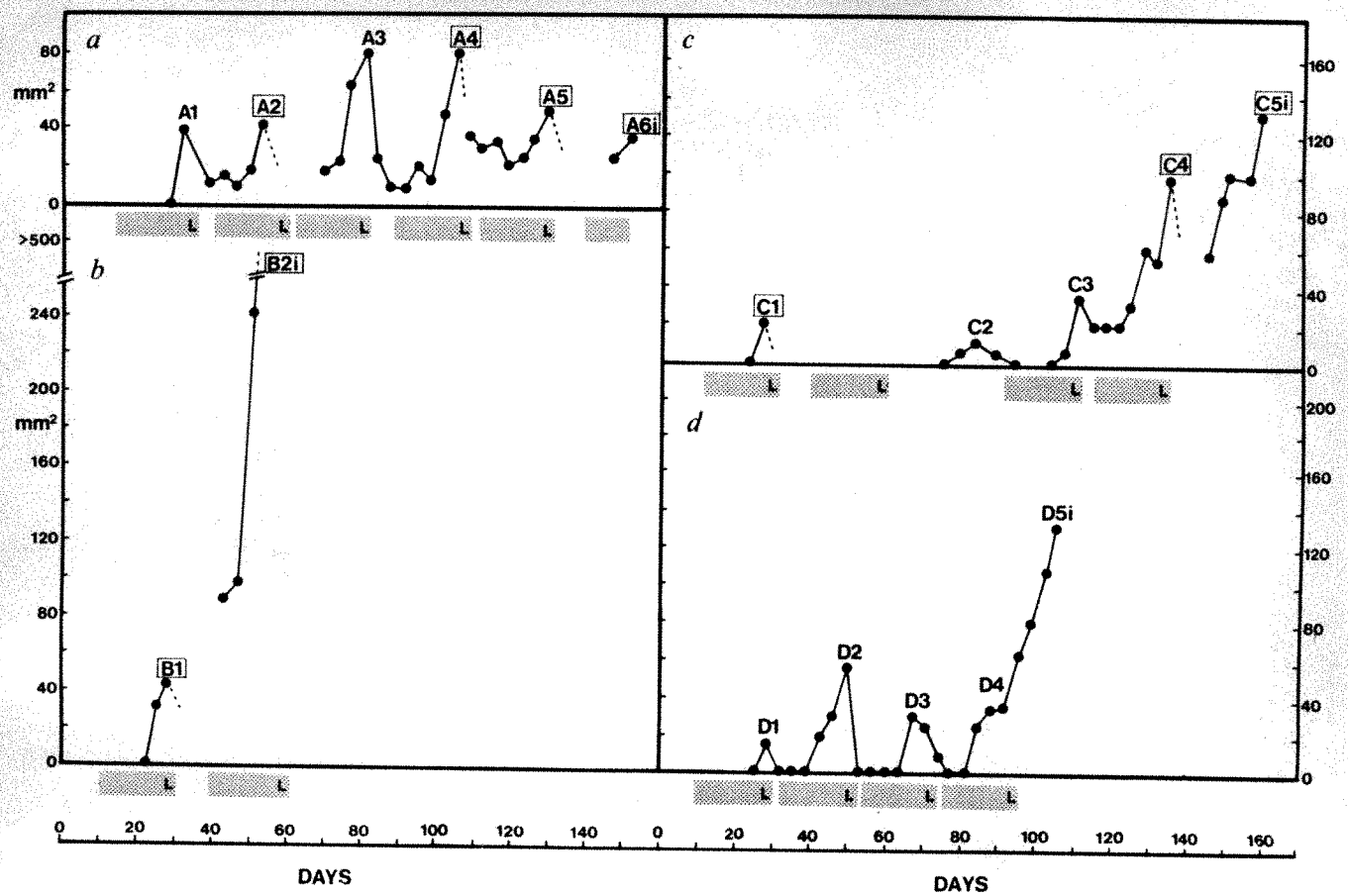


Fig. 1 Cycles of pregnancy-dependent tumour growth and regression. Female BR6 mice were routinely examined for palpable mammary tumours and the progression of each tumour during cycles of pregnancy was monitored. *a–d*, Four independent sites (A–D) at which recurrent tumours arose in four separate mice. The shaded bars below the abscissa of each panel indicate the observed periods of pregnancy, with L signifying delivery of a litter. Size measurements were taken twice weekly, and are expressed on the ordinate as mm^2, representing the product of two orthogonal diameters[8]. Boxed letters and numbers refer to the site and cycle of growth at which surgical biopsies, amounting to ~50% of the tumour mass, were removed for histological examination[9] and DNA extraction[2]. No biopsies were taken from site D in order to display a more typical pattern of tumour growth and regression. The symbol i indicates the cycle or biopsy at which the tumour was judged to have become hormone-independent. We are not certain that the tumour designated A6i had fully progressed to hormone independence since the presence of other independent tumours in the same mouse necessitated death at this point.

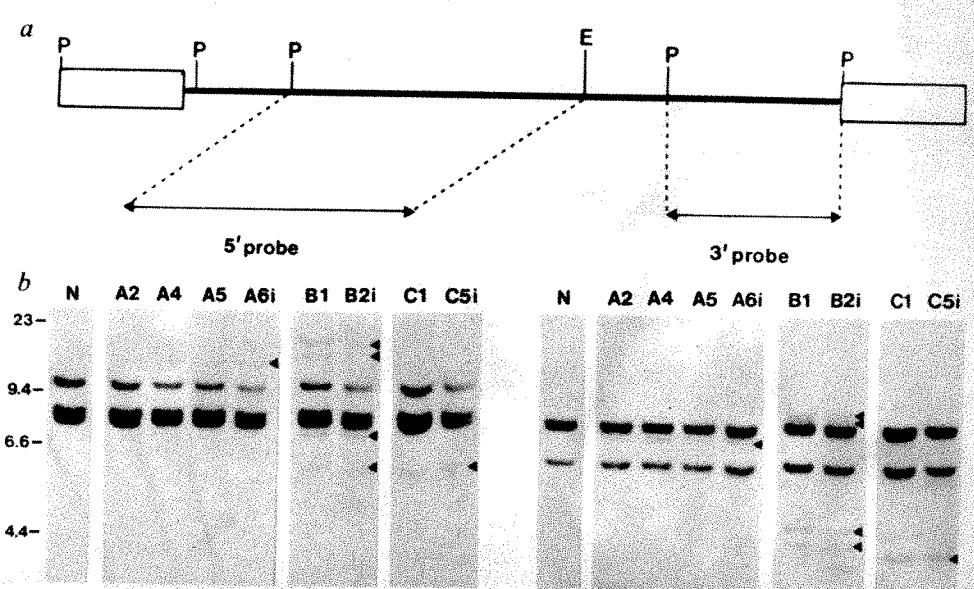


Fig. 2 Analysis of MMTV proviral units in recurring, pregnancy-dependent mammary tumours. *a*, A restriction map of the GR strain of MMTV provirus is depicted showing the sites of cleavage for the enzymes *Eco*RI (E) and *Pst*I (P)[10]. Digestion of cellular DNA with *Eco*RI generates two virus–cell junction fragments distinguishable by hybridization with the cloned 5′-specific and 3′-specific fragments as indicated[2]. *b*, DNA of high molecular weight was prepared[2] from freshly dissected biopsies of mammary tumours which recurred at three independent sites (A, B and C) in three different animals (see Fig. 1). The numbers refer to cycles of tumour growth, with i signifying the final, hormone-independent tumour. N represents the normal tissue control, in this case the spleen taken from the same animal as the A series of tumours. Samples of DNA (10 μg) were digested with an excess of *Eco*RI, fractionated on 0.8% agarose gels and transferred onto nitrocellulose filters as described previously[2]. The filters were then hybridized for 36–48 h with either the 5′ or 3′ specific fragment of viral DNA, labelled with ^{32}P-dCTP by nick-translation. Before autoradiography the filters were washed in 0.1 × SSC/0.1% SDS at 65 °C[2]. The sizes of the various virus–cell junctions detected in this way were estimated relative to fragments of λ DNA generated by digestion with *Hin*dIII (shown in kilobases on the left of the panel). Normal tissues from BR6 mice contain three endogeneous units of MMTV, characterized by 5′ junction fragments of 7.7, 7.9 and 9.6 kb, while the 3′-specific junctions appear as a 6.2 kb band and a 7.6 kb doublet. The positions of novel fragments corresponding to acquired MMTV proviruses are indicated (◄).

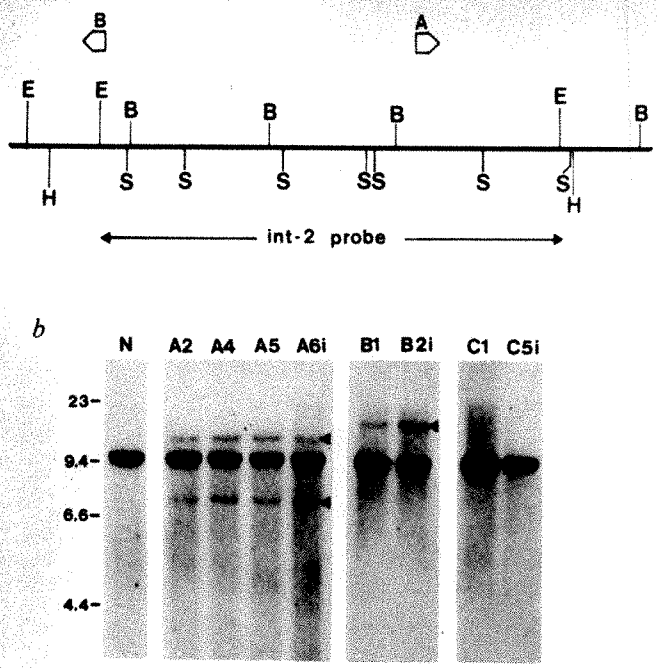


Fig. 3 Provirus insertion in the *int-2* locus in pregnancy-dependent mammary tumours. *a*, The restriction map of the MMTV *int-2* locus in BALB/c DNA indicates the sites of cleavage for the enzymes *Eco*RI (E), *Hin*dIII (H), *Bam*HI (B) and *Sac*I (S). The identification and characterization of this locus, and the derivation of the 10.0 kb *Eco*RI fragment used as a probe, have been presented elsewhere[2,4]. Using these enzymes, no restriction site polymorphisms were detected in the relevant DNAs from BALB/c and BR6 mice. The arrow symbols above the map indicate sites and orientation of integrated proviruses as discussed below. *b*, The nitrocellulose filter carrying the DNA samples analysed with the 3′-specific viral probe in Fig. 2*b* was rinsed in alkali to remove residual probe and then hybridized with the 10.0 kb *int-2* probe indicated. Conditions and symbols are as described in the legend to Fig. 2. N represents a sample of normal tissue DNA, in this case the spleen, showing the homologous uninterrupted 10.0 kb *Eco*RI fragment detected with this probe. The sizes of any novel fragments generated by provirus insertion (identified by the symbol ◄) were estimated relative to *Hin*dIII digested λ DNA standards as shown. From these data, it was possible to deduce both the site and transcriptional orientation of the provirus integrated within *int-2* in both the A and B tumour series. As indicated in *a*, these two proviruses map about 6-7 kb apart in the cellular DNA and are in opposite transcriptional orientations. In the C series of tumours, no provirus insertions were detected in over 30 kb of DNA centred on the *int-2* locus.

integration (as at tumour site A), we would argue that the acquisition of further proviral elements during progression of the tumour probably reflects fortuitous superinfection and does not contribute to the phenotypic change.

We have previously identified a region of DNA on mouse chromosome 7, *int-2*, in which an MMTV provirus has integrated in ~50% of all BR6 mammary tumours examined whether hormone-dependent or independent[2,4,14]. As we were interested in determining the status of this locus in the pregnancy-dependent tumour series, the nitrocellulose filters used in preparing Fig. 2 were rehybridized with a probe corresponding to the 10.0 kilobase (kb) *Eco*RI fragment from *int-2* (ref. 2). As indicated in Fig. 3, both the A and B tumour series showed evidence for provirus integration within this fragment. The simplest example is provided by the A series in which two novel *Eco*RI fragments were detected in addition to the normal 10.0 kb fragment present on the uninterrupted chromosome. The larger of these novel fragments was identical in size (~12.5 kb) to the virus–cell junction detected with a 5′-specific viral probe (Fig. 2*b*); while the smaller (~7.4 kb) coincided with the corresponding 3′-specific junction. These data are therefore consistent with integration of a complete MMTV provirus at the position, and in the transcriptional orientation, indicated on the restriction map in Fig. 3. With the site B tumours only one novel fragment was detected in these analyses, corresponding in size to one of the 5′-specific junctions observed in Fig. 2*b*. The provirus integration site therefore mapped very close to the 5′ boundary of the 10.0 kb *Eco*RI fragment, and in the orientation shown (Fig. 3*a*), such that the 3′-specific junction had only a very short region of overlap with the probe. This interpretation has been verified by preparing Southern blots with each of the different restriction endonucleases whose sites are depicted in Fig. 3*a* (data not shown).

Two significant conclusions can be drawn from these observations. First, the A and B tumour series are clonal, in that the same provirus, integrated within *int-2*, remains detectable and unaltered during cycles of pregnancy-dependent growth and regression and in the transition to hormone independence. The second is that since integration within *int-2* apparently occurs by the earliest appearance of the tumour, it may represent an important step in the development of neoplasia. This latter conclusion is supported by data to be presented elsewhere which indicate that polyadenylated RNA transcripts derived from the *int-2* locus are present specifically in tumours which have sustained a provirus integration in *int-2*. Our present interpretation is therefore that MMTV-induced carcinogenesis involves proviral activation of specific cellular genes and that *int-2* represents one such gene.

The observations of provirus insertions in these tumours make significant predictions regarding the involvement of *int-2* in tumorigenesis. If the activation of RNA and protein synthesis from a putative oncogene within *int-2* is the primary event in the onset of neoplasia, then either the expression of this gene product or the target to which its activity is directed, must remain under hormonal control. It is not possible at this stage to determine whether this reflects the direct action of hormones on some functions of the provirus[15-18], or whether the tumour cells are simply continuing to respond normally to the hormonal fluxes accompanying pregnancy[5,19]. However, the data also imply that activation of *int-2* or other analogous loci are not sufficient for the development of frank malignancy and may therefore represent only part of a multistage process. Some additional stochastic event, such as the activation or mutation of alternative cellular genes, may be required to obviate this hormone dependence and permit the tumour to develop autonomously. Pregnancy-dependent tumours in the BR6 mouse provide a powerful system in which to dissect the roles of these various factors in mammary carcinogenesis.

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Long terminal repeats of human T-cell leukaemia virus II genome determine target cell specificity

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Human T-cell leukaemias and lymphomas associated with the human T-cell leukaemia viruses (HTLV)[1-3] are invariably neoplasms of cells with mature T-lymphocyte phenotype[4,5]. Epstein–Barr virus-transformed B-lymphocyte lines which are productively infected with HTLV may be isolated from patients with HTLV malignancies[6,7], but no non-lymphoid tissues seem to be involved. Here, to investigate the basis for this tissue specificity, we introduced type II HTLV (HTLV-II) into a variety of human cells by infection and also by transfection of recombinant genomes. We found no HTLV-II expression in non-lymphoid tissues although expression and correct initiation of transcription was observed in B and T lymphocytes. Our results using recombinant genomes indicate that the restriction of expression is at least partly due to *cis*-acting functions of the long terminal repeats which lie at each end of the HTLV genome.

Normal human peripheral blood T lymphocytes and Epstein–Barr virus (EBV) transformed B-lymphocyte cell lines were productively infected by HTLV-II by co-cultivation with HTLV-II-infected Mo cells[7] but attempts to infect cells of non-lymphoid origin were unsuccessful (Table 1). Epithelial/B-lymphocyte hybrid cells (D98/Raji[8] and D98/HRI[9]) were infected, as shown by the presence of HTLV-II proviral DNA in ~10% of the cells for up to 50 generations, but no viral p19 or p24 antigens were detected by indirect immunofluorescence (Table 1) and co-cultivation of infected hybrid cells with normal human peripheral blood lymphocytes did not result in HTLV-II transformation of the normal cells (data not shown).

Table 1 HTLV-II infection by co-cultivation with Mo cells

Cells	HTLV-II genome	Viral antigens p19 and p24
Peripheral blood lymphocytes	+	+
EBV-transformed B cell		
G-B	+	+
WIL-2	+	+
Primary fibroblasts		
R	NT	–
C	NT	–
K	NT	–
Fibroblast cell line (143)[8]	NT	–
Epithelial/B-lymphocyte hybrid line		
D98/RAJI[9]	+	–
D98/HRI[11]	+	–

In every case 5×10^5 cells were co-cultivated with an equal number of lethally irradiated Mo cells[7]. In the case of adherent cells, the Mo cells were washed out after 1 week of co-culture and the cells passaged for at least an additional 2 weeks before the cells were fixed for indirect immunofluorescence for viral p19 and p24 antigens and their DNA extracted for hybridization by the method of Southern[18]. In the case of nonadherent cells, the cells were co-cultured for 5–6 weeks. Most Mo cells were dead by the second week of co-culture. NT, not tested.

Since HTLV-II infection by co-cultivation is a relatively inefficient process, probably due to the low titres of infectious virus produced by infected cells, we also used DNA transfection with selectable markers to introduce HTLV-II DNA into cells. We initially tested the ability of an HTLV-II provirus (λH6,

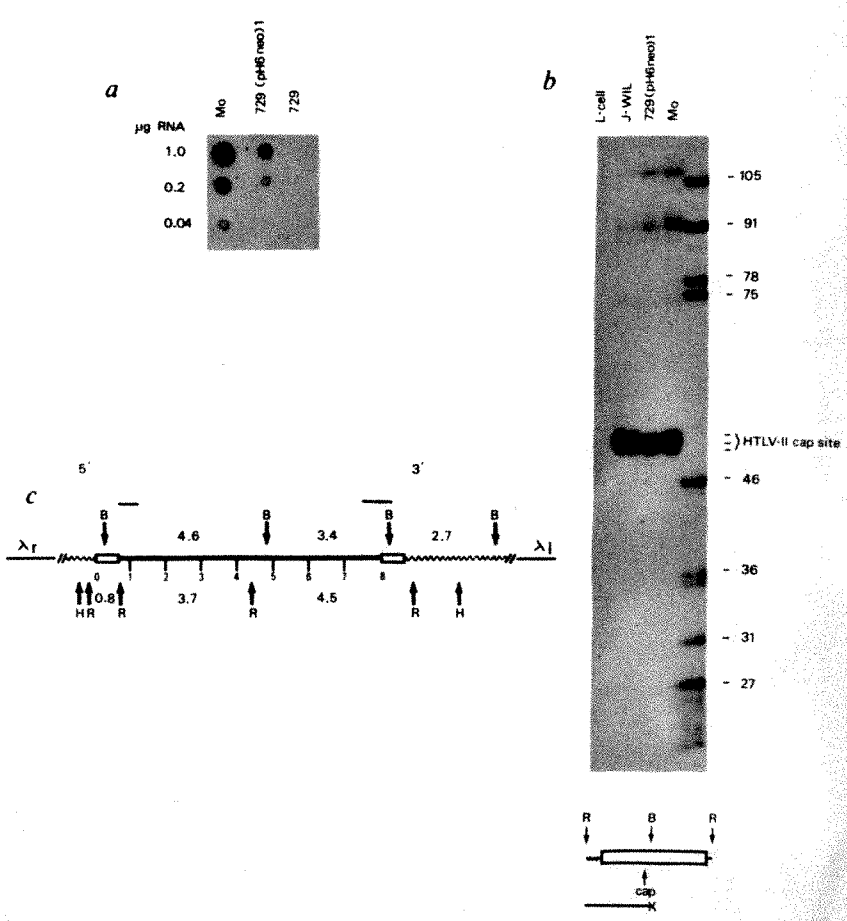


Fig. 1 HTLV-II RNA sequences in 729 B cells stably transfected with λH6 DNA. ***a***, Serial dilutions of poly(A)-containing RNA prepared from Mo cells; a clone of 729 cells transfected with pH6neo (729(pH6neo)1) and normal 729 cells. The indicated amounts of RNA were spotted onto nitrocellulose filter paper and hybridized[24]. The location of sequences in the probe is shown in *c* and are derived from cDNA clones pH-1 and pH-13[13]. The probe was purified free of pBR322 sequences so only HTLV-II-specific hybridization would be detected. ***b***, S_1 nuclease analysis[25] of total RNA from mouse L-cells; HTLV-II infected B cells (J-WIL); 729(pH6neo)1; and Mo cells. The cap site of HTLV-II (nucleotide 314 ± 1) was determined previously[20]. The position of the probe in the 5′ LTR of the λH6 provirus is indicated below the autoradiogram. The probe is 5′ end-labelled at the *Bam*HI site (nucleotide 360[20]) in the LTR. The expected sizes of the protected fragments are 50, 51 and 52 bases[20]. The size of *Sau*3A-digested pBR322 fragments are indicated. ***c***, The restriction enzyme sites *Hin*dIII (H), *Bam*HI (B) and *Eco*RI (R) in λH6 proviral DNA[13] and flanking human cellular DNA. The orientation of the provirus is indicated by 5′ and 3′ and the right and left arms of the λ1059 vector are indicated by λ_r and λ_l respectively. The numbers directly beneath the genome indicate the size in kilobases (kb). The numbers between restriction enzyme sites represent the size of HTLV-II DNA fragments. The location of HTLV-II sequences used in the hybridization probe are shown by bars indicated above the map.
Methods: 10^7 729 cells were transfected by spheroplast fusion[26] with a provirus HTLV-II subcloned in the *Eco*RI site of pSV2-neo[10] (pH6neo) and plated at 10^5 viable cells (viability was usually ~50%) per well of a 24-well culture dish. The frequency of G418-resistant cells was about 10^{-5}. Cells resistant to 750 μg ml^{-1} G418 were propagated for analysis. Details of the construction of pH6neo are available on request.

Fig. 1*c*) to function in an EBV-transformed B-cell line, 729, found to be competent for transfection by protoplast fusion. Cells were transfected with the entire provirus of λ H6 subcloned into pSV2-neo (ref. 10), a recombinant vector selectable in animal cells with the aminoglycoside antibiotic G418.

Approximately 25% of the G418-resistant clones of cells expressed HTLV p19 and p24 antigens as assayed by indirect immunofluorescence. Dot blot hybridization of RNA from one of these clones demonstrated expression of HTLV-II RNA (Fig. 1*a*) and S_1 nuclease analysis showed correct initiation from the cap site of the HTLV-II long terminal repeat (LTR) (Fig. 1*b*).

To study the restriction of HTLV-II expression in non-lymphoid cells, cells from a fibroblast cell line, 143, derived from a human osteosarcoma and competent for stable transfection[11], were transfected with λ H6 DNA. Since 143 cells are deficient in thymidine kinase, we used the co-transfection technique[12] with the herpes simplex virus thymidine kinase gene (pTK). In this way we obtained a stable 143 cell line containing the λ H6 provirus. Restriction enzyme analysis indicates that there are two HTLV-II DNA fragments in the cells, the smaller being the λ H6 provirus with no gross alterations of viral sequences (Fig. 2*a*). Other clones of 143 cells were obtained by transfection with a molecular clone of an HTLV-II provirus isolated from a transformed cell line, J-LB-II[7,13].

The 143 clone containing HTLV-II was negative for viral p19 and p24 antigens as assayed by indirect immunofluorescence. Poly(A)$^+$ RNA extracted from the cloned 143 cells did not hybridize to a HTLV-II-specific probe although hybridization could be detected in as little as 1.6 ng of RNA from HTLV-II-transformed Mo cells (Fig. 2*b*). Therefore, although the complete HTLV-II genome is present in the 143 cells, stable HTLV-II RNA is not detectable.

The lack of HTLV-II expression in several different human epithelial and fibroblast cells containing infected or transfected viral genomes indicates a tissue-specific restriction for HTLV-II transcription. Since the retrovirus LTR contains sequences important for the control of viral transcription and replication[14,15] we constructed recombinant genomes containing the HTLV-II LTR and the selectable gene *neo*^{R} from the bacterial transposable element Tn*5* used in pSV2-neo[10]. The transfection of selectable markers, the expression of which is dependent on *cis*-acting transcription signals, has been used as a simple assay for the efficiency of transcription of various viral and cellular promoters[16,17].

Four types of recombinant genomes were constructed to test the transcriptional efficiency of the HTLV-II LTR relative to the SV40 early promoter and SV40 termination sequences (Fig. 3). The first recombinant genome consisted of the *neo*^{R} gene, transcription of which would be initiated by the 5′ LTR, but termination of transcription would occur in SV40 sequences (pJH9LTRneo). The second recombinant represented the reciprocal of the first recombinant, in that the SV40 early promoter would initiate transcription of the *neo*^{R} gene and termination of transcription would occur in the 3′ LTR (pH6SVneoB). The third recombinant would initiate and terminate transcription of *neo*^{R} in the HTLV-II LTRs (pJH9LTRneoH6LTR). The fourth construct served as a control and consisted of the complete pSV2-neo plasmid inserted into the HTLV-II genome at the same position as for pH6SVneoB (pH6SVneo$^+$). Transcription of the *neo*^{R} gene would be initiated by the SV40 early promoter and terminated by SV40 sequences.

When the recombinant genomes were introduced into the Mo cells by protoplast fusion, G418-resistant cells were obtained at a frequency comparable to that of the SV40 vector, pSV2-neo (Fig. 3). No G418-resistant cells were obtained when Mo cells were fused with pBR322. In pJH9LTRneo and pJH9LTRneoH6LTR, expression of *neo*^{R} should be dependent upon the initiation of transcription by the HTLV-II LTR. Figure 4 shows an example of one Mo cell clone transfected with pJH9LTRneo. A DNA fragment containing *neo*^{R} is detected in the Mo cells by Southern hybridization[18]. The size of the fragment is consistent with the presence of the entire transcriptional

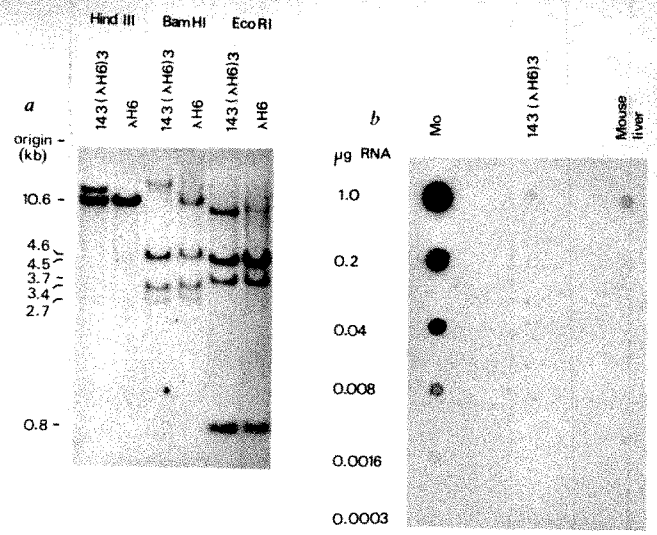


Fig. 2 HTLV-II DNA and RNA sequences in 143 cells stably transfected with λ H6 DNA. ***a***, DNA extracted from the tk$^+$ transformant 143(λ H6)3 digested with *Hin*dIII, *Bam*HI and *Eco*RI and hybridized by the method of Southern[18] using the probe shown in Fig. 1*c*[13]. λ H6 DNA is equivalent to 1.0 copy per cell mixed with 15 μg of normal human DNA and treated in parallel. As *Hin*dIII does not cleave HTLV-II sequences[13] the two fragments detected by *Hin*dIII digestion must represent two HTLV-II DNA fragments present in the cells. The smaller of the two *Hin*dIII fragments is the same size as the 10.6 kb *Hin*dIII fragment of λ H6. The larger fragment has lost several of the restriction enzyme sites of λ H6 and contains only a portion of the λ H6 provirus. The HTLV-II DNA fragments indicated in kb are those shown on the map of λ H6 in Fig. 1*c*. ***b***, Poly(A)$^+$ RNA prepared from Mo, 143(λ H6)3 and mouse liver cells and treated as described in Fig. 1*a*. Because of the long exposure of the autoradiogram, some nonspecific hybridization to mouse liver RNA is evident.

Methods: 5×10^5 tk$^-$ 143 cells[11] were co-transfected with a calcium phosphate precipitate of 0.5 μg pTK, 1 μg λ H6[13] and 20 μg ml^{-1} calf thymus DNA. Colonies of cells resistant to hypoxanthine–aminopterin–thymidine medium were cloned after 14 days and propagated.

unit of *neo*^{R} (Fig. 4*a*). In addition, protection of a 790 nucleotide DNA fragment by S_1 analysis (Fig. 4*b*) is consistent with initiation of *neo*^{R} transcription at the cap site of the HTLV-II LTR. Other Mo clones transfected with pJH9LTRneo and pJH9LTRneoH6LTR show the same 5′ ends (data not shown). Therefore, transcription of *neo*^{R} in Mo cells appears to be dependent upon HTLV-II LTR function.

Transfection into 143 fibroblast cells of pSV2-neo, where SV40 sequences are used for initiation and termination of *neo*^{R} transcription, resulted in approximately 200 colonies of G418-resistant cells per μg of plasmid (Fig. 3). However, transfection of recombinant genomes where the HTLV-II LTR was necessary for initiation of transcription (pJH9LTRneo, pJH9LTRneoH6LTR) resulted in less than 1/100th the number of colonies. The efficient transfection in 143 cells of pH6SV2neo$^+$, where expression of *neo*^{R} is dependent upon SV40 signals contained between HTLV-II LTR sequences, indicates that viral sequences are not toxic to 143 cells and are not inhibitory to uptake or stable integration of transfected DNA. Therefore, the LTR of HTLV-II appears not to promote the initiation of transcription of the *neo*^{R} gene in 143 cells effectively.

Note, in addition, that although the SV40 early promoter is functional in 143 cells when transcription is terminated in SV40 sequences (pSV2-neo), the SV40 promoter appears to be incompatible with the 3′ HTLV-II LTR when the LTR is substituted for SV40 termination sequences (Fig. 3, pH6SVneoB). In 143 cells this recombinant had a transfection efficiency that was no better than a recombinant with no termination sequences (Fig. 3, pSVneoA-). In Mo cells, pH6SVneoB had a somewhat lower efficiency of transfection than pSV2-neo but higher than

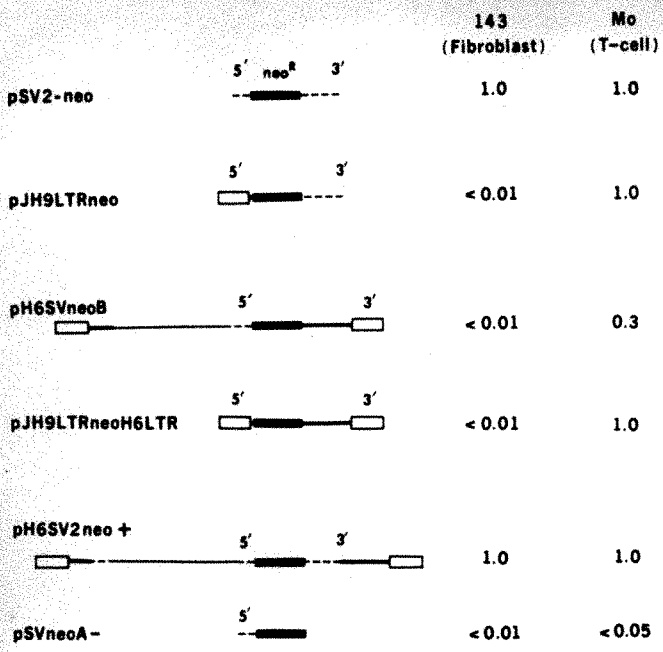


Fig. 3 Relative transfection efficiency of recombinant *neo*^{R} genes. The different recombinants between SV40, HTLV-II and *neo*^{R} sequences are shown schematically. HTLV-II sequences are indicated by dark lines; HTLV-II LTR by open boxes; SV40 sequences by dotted lines; *neo*^{R} sequences by solid bars and pBR322 sequences by thin lines. 5′ and 3′ indicate the orientation with respect to expected transcription initiation sequences and termination sequences, respectively. The 5′ LTR in pJH9LTRneo and pJH9LTRneoH6LTR are joined to the *neo*^{R} gene by an *Eco*RI site which is located 3 bp from the tRNA^{Pro} primer binding site[20]. All constructs are propagated as plasmids of pBR322 derivatives. Details of the constructs are available on request.

Methods: 143 cells[11] were transfected by the calcium phosphate precipitation method with serial fivefold dilutions of the plasmid DNA. Colonies of cells resistant to 200 μg ml^{-1} G418 were counted after 2 weeks. 10^7 Mo cells were transfected by the spheroplast fusion method[26] and plated at $1.0–3.0 \times 10^5$ viable cells (viability ~50%) per well of a 24-well culture dish. The number of stably transfected cells resistant to 500 μg ml^{-1} G418 was determined by the proportion of wells in which cells grew after 5–6 weeks of culture. The efficiency of transfection using the SV40 vector pSV2-neo[10], which contains the SV40 early promoter and termination sequences, was about 5×10^{-6}. Because of some variability between transfection experiments performed at different times, the efficiency of transfection was determined relative to pSV2-neo in each experiment.

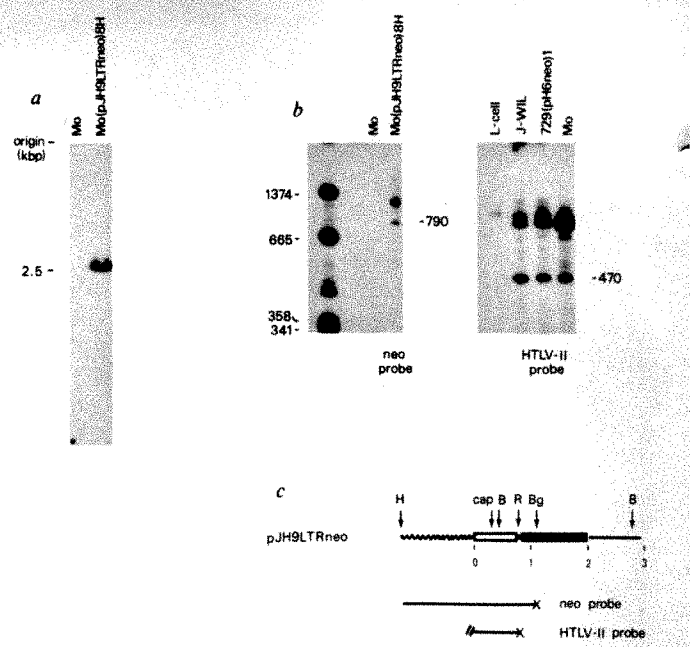


Fig. 4 pJH9LTRneo DNA and RNA sequences in a clone of transfected Mo cells, Mo(pJH9LTRneo)8H. *a*, DNA from Mo(pJH9LTRneo)8H digested with *Bam*HI and hybridized[18] with a ^{32}P-labelled DNA fragment consisting of only *neo*^{R} sequences. The DNA fragment of 2.5 kb is the expected size for the *Bam*HI fragment of pJH9LTRneo (see map in *c*). *b*, S_1 nuclease analysis of *neo*^{R} containing RNA in Mo(pJH9LTRneo)8H (left panel) using a 5′ end-labelled *neo*^{R}-specific probe. S_1 nuclease analysis of HTLV-II RNA (right panel) using an HTLV-II specific probe is shown for comparison. The protected DNA fragments indicated, 790 and 470 nucleotides, are of the size expected for transcription initiation from the cap size of the HTLV-II LTR. The larger protected fragment with RNA from Mo(pJH9LTRneo) results from protection of the entire HTLV-II LTR sequences (beyond the cap site to the 5′ end). This protection is a consequence of hybridization of the U3R sequences at the 3′ end of HTLV-II RNA to the U3R LTR sequences in the probe thereby further protecting the probe to the 5′ end of the LTR. More sensitive mapping with shorter probes showed that the precise 5′ end of the LTR is protected (data not shown). Similar larger protected DNA fragments are observed with the HTLV-II specific probe (right panel, middle band). The largest fragment observed with the HTLV-II specific probe is the size of the full length probe, a result of incomplete S_1 digestion and also visible in the L-cell control lane. *c*, Schematic map of pJH9LTRneo. Symbols are as in Fig. 3 except that wavy lines represent human cell DNA sequences. The 5′ end-labelled probes for S_1 analysis are shown. The HTLV-II probe is derived from the equivalent region of the 5′ LTR of λH6 (Fig. 1*c*) up to the 5′ *Eco*RI site in human cell DNA. The numbers beneath the map indicate the size in kb. The cap site of HTLV-II is indicated. H, *Hin*dIII; R, *Eco*RI; Bg, *Bgl*II; B, *Bam*HI.

pSVneoA-. Thus, termination of transcription by the HTLV-II LTR may also be restricted in 143 cells.

Our results demonstrate that in 143 fibroblast cells the HTLV-II LTR functions poorly, if at all, to promote transcription of stable mRNA, in contrast to the Mo cells where the LTR functions efficiently. This pattern parallels that of expression of HTLV-II proviral sequences in 143 and Mo cells (Fig. 1). Note that other retroviruses, Kirsten murine leukaemia–sarcoma virus and simian sarcoma-associated virus, have been shown to replicate in 143 cells[19]. Therefore, a *cis*-acting property of the HTLV-II LTR appears to be directly responsible for the lack of HTLV-II expression in the non-lymphoid cells tested.

We recently determined the sequence of the HTLV-II LTR[20], and found a sequence of 21 nucleotides, repeated three times in the same orientation, present in U3 upstream from the 'TATAA' box. Although most sequences of the adult T-cell leukaemia virus (HTLV-I) LTR[21] are distinct from those in HTLV-II, we noted that the 21 nucleotide sequence is highly conserved and repeated in comparable positions of U3. Because this repeated sequence is conserved in regions of the LTR important for transcriptional control, it is possible that this sequence is required as a *cis*-acting signal for the tissue specificity of LTR function.

The specific transcription of HTLV-II sequences in lymphoid cells resembles the specific transcription of immunoglobulin genes in mouse B lymphocytes[16,22,23]. Transfected immunoglobulin genes are expressed at low levels in fibroblasts in contrast to their expression in mouse myeloma cells. Sequences related to enhancer sequences of animal viruses have been shown to be responsible for this tissue-specific expression. It is possible that the factors which control the tissue-specific expression of HTLV and immunoglobulin genes represent basic mechanisms for controlling gene expression during T- and B-lymphocyte development respectively. HTLV has apparently evolved in such a manner as to use that mechanism for its pathogenesis in human T lymphocytes.

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Domain interactions of H–2 class I antigens alter cytotoxic T-cell recognition sites

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H–2 class I antigens appear to direct the recognition of virus-infected and neoplastic transformed cells by cytotoxic T lymphocytes (CTLs)[1,2]. Here, to identify the regions of class I antigens involved in CTL recognition, four hybrid class I genes were constructed in which exons were exchanged between the *H–2K*^{b} and *H–2D*^{b} genes[3,4]. These class I genes were expressed in mouse L cells and recognition of the hybrid K^b/D^b antigens by CTLs and monoclonal antibodies specific for either K^b or D^b was investigated[4]. The pattern of CTL and monoclonal antibody recognition obtained indicates three correlations between structure and function of class I antigens. First, most CTL recognition sites and alloantigenic determinants are located on domains 1 and 2 of the antigen molecule. Second, these CTL recognition sites and alloantigenic determinants are not influenced by interaction of domains 1 and 2 with polymorphic regions of domain 3. Third, in contrast, interaction between domains 1 and 2 alters these CTL recognition sites and alloantigenic determinants. The alteration of CTL recognition sites by interaction between domains 1 and 2 suggests that a CTL site may be formed by amino acids from both domains 1 and 2, or that the conformation of amino acids at a CTL site may be altered by interactions between domains 1 and 2. Through these two features, the conformation of CTL recognition sites on H–2 class I antigens may be sensitive to alteration by interaction of either domain 1 or 2 with viral antigens.

The four hybrid class I genes constructed consist of two pairs of reciprocal exon exchanges between the *H–2K*^{b} and *H–D*^{b} genes (Fig. 1*a*). Thus, exons 2 were exchanged: the resultant constructs of *K*^{b} exon 2 in the *D*^{b} gene and *D*^{b} exon 2 in the *K*^{b} gene encode hybrid K^b/D^b antigens consisting respectively of a polypeptide with domain 1 of K^b and all other domains from D^b (K^{b}1/D^b) and a polypeptide with domain 1 of D^b and all other domains from K^b (D^{b}1/K^b) (Fig. 1*b*). Similarly, the promoters and exons 1, 2 and 3 were exchanged: the resultant constructs of the *K*^{b} promoter and exons 1, 2 and 3 spliced to the remainder of the *D*^{b} gene and the *D*^{b} promoter and exons 1, 2 and 3 spliced to the remainder of the *K*^{b} gene encode hybrid K^b/D^b antigens, producing, respectively, K^b domains 1 and 2 with all other domains from D^b (K^{b}1+2/D^b) and D^b domains 1 and 2 with all other domains from K^b (D^{b}1+2/K^b) (Fig. 1*b*). Ltk$^-$ cells were co-transformed with the *K*^{b}, *D*^{b} and four *K*^{b}/*D*^{b} hybrid genes and the thymidine kinase (*tk*) gene by $CaPO_4$ co-precipitation[5]. Ltk$^+$ colonies, selected for by growth in the presence of hypoxanthine–aminopterin–thymidine, were picked and screened by antibody binding for cell-surface expression of K^b and/or D^b alloantigenic determinants. The cloned cell lines expressing high levels of K^b, D^b and hybrid K^b/D^b antigens were selected for further study.

The presence or absence of K^b and D^b allodeterminants on the four hybrid K^b/D^b antigens was determined by screening these cell lines with a panel of 18 monoclonal antibodies specific for determinants on either K^b or D^b (Table 1). All these allodeterminants, except one D^b allodeterminant, map to domain 1 or 2. The specificities of 12 monoclonal antibodies were mapped to either domain 1 or 2; these locations were basically in agreement with clusters of allodeterminants defined by binding inhibition studies[6]. The D^b-specific antibody 28-14-8 mapped to domain 3; the allodeterminant recognized by 28-14-8 was also present on L^d and has previously been mapped to domain 3 of this antigen[7].

The conformation of domains 1 and 2 in the hybrids K^{d}1/D^b and D^{b}1/K^b seems to differ from that in the parental K^b and D^b antigens. Fifteen out of the 17 parental K^b and D^b alloantigenic determinants located on domains 1 and 2 are either modified or not present on K^{b}1/D^b and D^{b}1/K^b. Thus, five allodeterminants are absent from either of the two hybrids; these are defined by antibodies K7-38, K9-136 and K10-56.1 specific for K^b and H141-29 and K15-25 specific for D^b. Ten allodeterminants are present on one or other of these hybrids, but all are modified, as indicated by markedly reduced levels of binding of the respective antibodies to the hybrids compared with K^b or D^b. The hybrids K^{b}1/D^b and D^{b}1/K^b, however, do retain one and two unaltered allodeterminants respectively, defined by antibody 28-13-3 specific for K^b and antibodies 28-14-8 and B22-249.1 specific for D^b; this indicates that both antigens are expressed at levels similar to K^b and D^b.

Recognition of the four K^b/D^b hybrid antigens by CTLs was investigated with both K^b and D^b restricted influenza virus-specific CTLs and K^b and D^b allospecific CTLs (Table 2, Fig. 2). The pattern of recognition obtained for the two hybrids K^{b}1+2/D^b and D^{b}1+2/K^b indicates first that the majority of both allospecific and virus-specific CTL recognition sites are on domains 1 and 2, and second, that these CTL recognition sites are not influenced by interaction of domains 1 and 2 with polymorphic regions of domain 3; the amino acid sequence of K^b differs from D^b at 9 residues out of 92 in domain 3 (refs 3, 8). These results for CTL recognition of the hybrids K^{b}1+2/D^b and D^{b}1+2/K^b confirm and extend those obtained for the equivalent L^d/D^d hybrids[9,10].

In contrast, neither the K^{b}1/D^b nor the D^{b}1/K^b hybrid was recognized by either K^b and D^b allospecific or K^b and D^b restricted influenza virus-specific CTLs. This indicates that both allospecific and virus-specific CTL recognition sites, located on domains 1 and 2 of the parental K^b and D^b antigens, are significantly altered on the K^{b}1/D^b and D^{b}1/K^b hybrid antigens. The cell lines 4-1 (which expresses K^{b}1/D^b) and 3-7 (D^{b}1/K^b) were lysed by an influenza virus-specific and K^k-restricted CTL clone (K5)[11] when infected with virus (data not shown). The lack of lysis of 4-1 and 3-7 by K^b and D^b specific CTLs therefore results from failure to recognize the hybrid antigens and not inability of the lines to be lysed.

Table 1 K^b and D^b specific monoclonal antibody binding to hybrid K^b/D^b antigens

Monoclonal antibody	K^b/D^b antigen Cell line	Antibody binding (c.p.m. ^{125}I bound) K^b 1-3	D^b 2-5	D^b1/K^b 3-7	K^b1/D^b 4-1	D^b1+2/K^b 6-2	K^b1+2/D^b 5-6	Location of allodeterminant
28-13-3		1,036	166	636	68	97	1,541	K^b2
K9-178		4,561	136	1,166	115	107	6,279	K^b2
K7-309		3,009	97	423	83	61	4,259	K^b2
K7-65		2,495	87	326	75	69	3,793	K^b2
20-8-4		5,004	96	81	2,308	40	5,829	K^b1
B8-3-24		3,880	96	57	1,239	90	5,044	K^b1
K7-38		1,203	189	90	217	93	1,931	K^b1 and/or 2
K9-136		1,659	69	91	142	64	2,013	K^b1 and/or 2
K10-56.1		4,700	89	163	85	55	6,172	K^b1 and/or 2
28-14-8		190	4,878	101	3,981	92	6,675	D^b3
T17.433		119	5,686	78	2,433	4,757	167	D^b2
28-11-5		155	1,859	67	550	1,827	119	D^b2
H172-93		114	1,809	81	302	1,459	77	D^b2
H166-32		194	2,114	149	325	1,710	148	D^b2
H141-31		124	2,073	87	292	1,622	86	D^b2
B22-249.1		189	5,745	4,180	86	5,674	55	D^b1
H141-29		97	639	72	115	671	63	D^b1 and/or 2
K15-25		144	340	124	118	317	109	D^b1 and/or 2

The K^b and D^b specific monoclonal antibodies have been characterized previously[6,13,14]. The antibody binding assay was performed in Falcon flat-bottom 96-well microtitre plates. 10^5 cells were added to each well and incubated overnight at 37 °C in Dulbecco's modified Eagle's medium supplemented with glutamine, hypoxanthine, aminopterin, thymidine, penicillin, streptomycin and 10% fetal calf serum. The cells were washed with phosphate-buffered saline containing Ca^{2+}, Mg^{2+} and 1% bovine serum albumin, and incubated with 50 μl per well of a 5× dilution of hybridoma supernatant for 60 min at 4 °C. After washing, the cells were incubated for 60 min at 4 °C with 50 μl per well of ^{125}I-labelled goat anti-mouse IgG containing 100,000 c.p.m. The cells were washed extensively and lysed with 1% Triton X-100; supernatants were collected and radioactivity was determined in a γ counter. Results are expressed as c.p.m. ^{125}I bound; each result is the average from triplicate samples and the variation in all cases was not >7.5%. The binding of an irrelevant monoclonal antibody, specific for the haemagglutinin of influenza virus A/PR8, was <150 c.p.m. on all six cell lines. All 18 K^b and D^b specific antibodies gave binding of <150 c.p.m. to a Ltk^+ cell line 8-1, which was obtained by transfection of Ltk^- cells with the *tk* gene alone. Three antibodies that react with determinants on $H\text{-}2^k$ class I antigens, H142-23.3, H142-45 and Y-3 (ref. 13), gave binding to the cell line 8-1 of 4,387, 551 and 6,147 c.p.m., respectively. This suggests that the level of expression of K^b, D^b and the four hybrid K^b/D^b antigens is similar to that of endogenous $H\text{-}2^k$ class I antigens of the L cells.

Thus, the pattern of CTL and monoclonal antibody recognition obtained for the two hybrid antigens, K^b1/D^b and D^b1/K^b, indicates that interactions between domains 1 and 2 of the hybrids alter parental K^b and D^b CTL recognition sites and alloantigenic determinants located on these domains. Two possible, not mutually exclusive, models would explain the alteration of CTL recognition sites and alloantigenic determinants. First, the sites recognized by CTLs or monoclonal antibodies may be formed by amino acids from both domains 1 and 2. Thus, for example, amino acids from D^b domain 1 that form part of a CTL/antibody site are replaced by different residues from K^b domain 1 in the hybrid K^b1/D^b. Second, the CTL/antibody sites may be formed by amino acids entirely from one domain. In this case, the conformation of a CTL/antibody site on D^b domain 2 in the hybrid K^b1/D^b, for example, would be altered by interaction of this domain with K^b domain 1. Thus, K^b differs from D^b at 13 amino acid residues out of 90 in domain 1 and at 20 residues out of 92 in domain 2 (refs 3, 8); these differences between K^b and D^b in domains 1 and 2 result in alteration of CTL/antibody sites on the hybrid antigens K^b1/D^b and D^b1/K^b.

Sherman[12] has proposed that H-2 restriction of CTLs may represent the recognition of conformational alterations of class I antigens resulting from interaction with viral antigens. Our report suggests that the conformation of a CTL recognition site

Table 2 Polyclonal allospecific and influenza virus-specific CTL recognition of hybrid K^b/D^b antigens

	K^b/D^b antigen Cell line	% Specific ^{51}Cr release (K:T = 20:1) K^b 1-3	D^b 2-5	D^b1/K^b 3-7	K^b1/D^b 4-1	D^b1+2/K^b 6-2	K^b1+2/D^b 5-6
CTLs							
Anti-A/X31 + D^b		−5	33	−4	7	22	2
Anti-A/X31 + K^b		18	1	−4	2	−1	13
Anti-allo D^b		−3	27	0	−3	24	2
Anti-allo K^b		34	−2	5	−2	−5	19

D^b and K^b restricted CTLs specific for influenza virus A/X31 were obtained from spleens of B10.HTG (K^dD^b) and B10.A(5R) (K^bD^d) mice respectively primed by intravenous injection with 10^7 A/X31 infected syngenic spleen cells. Allospecific CTLs were generated by *in vitro* stimulation of BALB/c (K^dD^d) spleen cells twice with irradiated (3,000 rad) B10.A(5R) (K^bD^d) for anti-K^b or twice with irradiated B10.HTG (K^dD^b) and once with irradiated B10.BYR (K^qD^b) spleen cells for anti-D^b. Ltk^+ target cells infected with influenza virus were prepared as follows: the cells were trypsinized and plated overnight on bacteriological Petri dishes, for easy resuspension, before infection with *A/X31* and ^{51}Cr-labelling for 1 h at 37 °C. The conditions for infection of the targets and the protocol for the 6-h cytotoxicity assay were as described previously[15]. The % virus-specific ^{51}Cr release was calculated by subtraction of ^{51}Cr release from uninfected targets. The % allospecific ^{51}Cr release was calculated by subtraction of ^{51}Cr release from a Ltk^+ cell line 8-1, which was obtained by transfection of Ltk^- cells with the *tk* gene alone. A CTL to target ratio (K:T) of 20:1 was used for all four polyclonal populations.

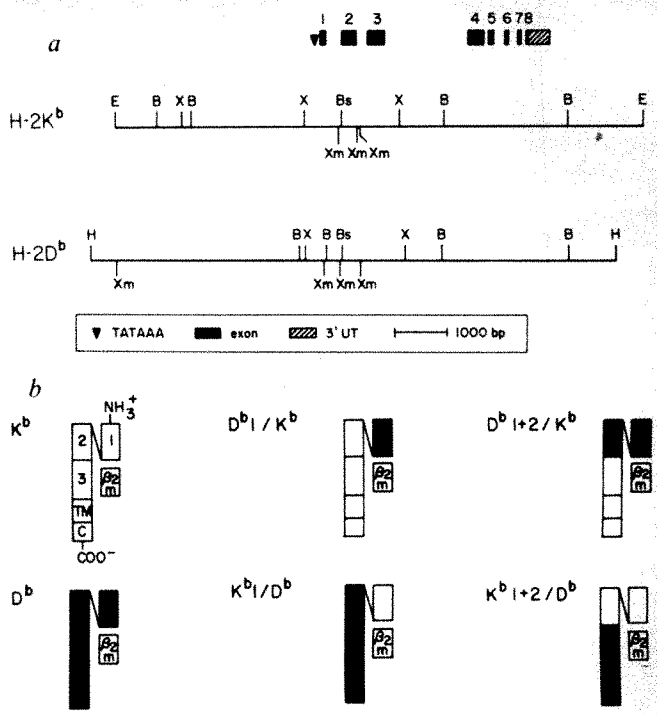


Fig. 1 *a*, Restriction maps of $H\text{-}2K^b$ and $H\text{-}2D^b$ genes. *b*, Domain structure of K^b/D^b and four K^b/D^b hybrid antigens. In *a*, E = *Eco*RI; B = *Bam*HI; Bs = *Bss*HII; H = *Hin*dIII; X = *Xba*I; Xm = *Xma*I. The restriction sites shown are those used to construct and confirm orientation of exon exchanges. The location of the promoter and exons of the K^b gene are shown[3]. Exons 1, 2 and 3 of the D^b gene are located in the 2,200-base pair (bp) *Bam*HI fragment that contains the *Bss*HII site. Exons 4, 5, 6, 7 and 8 are located in the adjacent 2,400-bp *Bam*HI fragment (unpublished data). The restriction sites used to isolate D^b exon 2 and exons 2 and 3 are not present in the nucleotide sequence of a D^b cDNA clone[3], which indicates that these sites are located in introns of the D^b gene. In *b*, 1, 2 and 3 are external domains 1, 2 and 3 (alternatively designated N, C1 and C2, respectively[16]); TM, transmembrane segment; C, cytoplasmic domain; β_2M, β_2-microglobulin. H-2 class I antigens are cell-surface glycoproteins of molecular weight (MW) 45,000 associated noncovalently with β_2-microglobulin, MW 12,000. The class I polypeptide can be divided on the basis of its primary structure into three external globular domains, each of about 90 amino acids, a transmembrane segment of ~40 residues, and one cytoplasmic domain of 30 residues[16]. The diagrammatic representation of the H-2 class I antigen is supported by the observation that domains 1 and 2 are stable in isolation and that domain 3 is associated with β_2-microglobulin[17].

Methods: The four hybrid K^b/D^b genes shown in *a* were constructed as follows: (1) Exchange of exon 2 between D^b and K^b. The K^b and D^b genes were subcloned in the *Eco*RI and *Hin*dIII sites of pBR327, respectively. The K^b and D^b exons 2 were isolated as *Xma*I 330- and 390-bp fragments, respectively; these fragments contain the *Bss*HII site. The D^b *Xma*I 390-bp fragment was inserted directly into a K^b gene subclone, linearized with *Xma*I, from which both *Xma*I fragments of 330 and 50 bp had been deleted. This deletion was obtained by complete digestion of the K^b subclone with *Xma*I and ligation at dilute concentration of DNA to favour recirculation of the vector minus the two fragments. The K^b *Xma*I 330-bp fragment was inserted first into a D^b 5′ gene subclone of the 2,200-bp *Bam*HI fragment, linearized with *Xma*I, from which the *Xma*I fragment of 390 bp had been deleted. This deletion was obtained by complete digestion of the D^b 5′ gene subclone with *Xma*I and ligation at dilute concentration of DNA. Second, a 1,200-bp *Xba*I–*Bss*HII fragment was isolated from the D^b 5′ gene subclone and inserted in the complete D^b gene subclone, linearized by partial digestion with *Xba*I and complete digestion with *Bss*HII. (2) Exchange of promoter and exons 1, 2 and 3 between K^b and D^b. The K^b and D^b promoters and exons 1, 2 and 3 were isolated from the subclones of the two genes as *Xba*I fragments of 1,800 and 1,850 bp, respectively. The corresponding K^b and D^b 3′ gene *Xba*I fragments of 8,000 and 11,500 bp, respectively, were isolated from the same digests; these fragments also contain the pBR327 vector sequence. The K^b *Xba*I 1,800-bp fragment was ligated to the D^b *Xba*I 11,500-bp fragment; similarly, the D^b *Xba*I 1,850-bp fragment was ligated to the K^b *Xba*I 8,000-bp fragment. Restriction enzyme digest conditions were as recommended by New England Biolabs. Restriction fragments were separated by electrophoresis in low melting temperature agarose gels; fragments were isolated by melting gel slices at 65 °C and phenol extraction. Purified fragments were ligated with T4 DNA ligase in 50 mM Tris *p*H 8.0, 7.5 mM $MgCl_2$, 1 mM ATP and 10 mM dithiothreitol for 3 h at 15 °C. Vector fragments were first treated with calf intestinal phosphatase in 50 mM Tris *p*H 9.0, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$ and 1 mM spermidine for 1 h at 37 °C. Ligated DNAs were introduced into $CaCl_2$-treated *Escherichia coli* DH1 or HB101 cells[18]. Colonies were picked and constructs were identified by restriction enzyme digests on plasmid mini-preps[18]. DNA for transfection of Ltk⁻ cells was isolated from plasmid maxi-preps[18]; exon exchange and correct orientation of insertion were confirmed by restriction mapping.

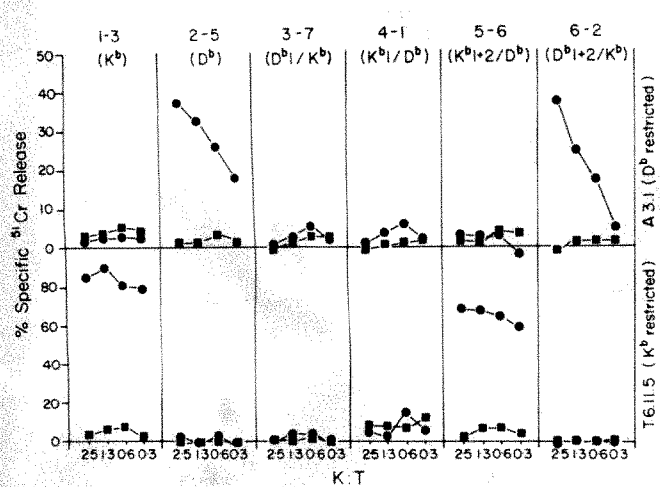


Fig. 2 Cloned influenza virus-specific CTL recognition of hybrid K^b/D^b antigens. The A/X31 influenza virus-specific CTL clones A3.1, D^b-restricted, and T6.11.5, K^b-restricted, were selected and maintained with antigen and T-cell growth factor as described previously[19]. Target cells were prepared and the 6-h cytotoxicity assay was performed as described in Table 2 legend. ●, A/X31-infected target cells; ■, uninfected target cells; K:T, CTL to target ratio. The upper graphs show lysis by D^b-restricted clone A3.1; the lower graphs show lysis by K^b-restricted clone T6.11.5.

may be sensitive to alteration by interaction of either domain 1 or 2 with a viral antigen.

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Two mechanisms of expression of a predominant variant antigen gene of *Trypanosoma brucei*

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African trypanosomes evade the host immune response by periodically switching their variant surface glycoprotein (VSG) coat[1–3]. The resulting, serologically distinct variant antigenic types (VATs) appear in a loosely ordered sequence and those arising early in infections are termed predominant VATs[4–8]. VAT switching reflects the successive transcriptional activation of single VSG genes from a large repertoire. Activation of some VSG genes is accomplished by duplication of a previously silent basic copy (BC) gene and insertion of this expression linked copy (ELC) near a chromosomal telomere where it is expressed[9–16]. However, other VSG genes, always located near a telomere, use a non-duplication activation (NDA) mechanism[16–19]. We report here that the gene encoding the predominant IsTat 1.A VSG can be activated with or without duplication. Four of six independently derived clones activated the 1.A gene without gene duplication or detectable rearrangement. The other two contained an active 1.A ELC, possibly generated by a gene conversion extending to the end of the telomere. The ability to utilize both NDA and ELC mechanisms of gene activation and perhaps an alternative mechanism for gene duplication may account for the fact that VAT 1.A is the most predominant VAT of the IsTaR 1 serodeme.

Derivation of the IsTaR 1 serodeme has been described previously[20]. Many immune competent rats, mice or *Peromyscus* singly infected with different IsTaR 1 VATs produced first relapse populations that were invariably 100% VAT 1.A. Thus, 1.A is the most predominant VAT of the IsTaR 1 serodeme. Each of six independently derived 1.A clones (Fig. 1) reacted with four monoclonal antibodies which recognize different epitopes of the 1.A VSG molecule (P.M., unpublished results), indicating that they all expressed the same VSG. The mechanism of 1.A VSG gene activation in these clones was examined by genomic Southern analyses. Figure 2*a* shows an autoradiogram of *Sal*I-digested DNA from five clones which do not express 1.A VSG (1.D, 1.3, 1.5, 1.7, 1.11), five 1.A clones from the first relapses of each of these ($1.A^1$, $1.A^3$, $1.A^5$, $1.A^7$, and $1.A^{11}$), a subclone of the original IsTat 1.A (1.Ae), and two clones derived from a first relapse population of IsTat 1.A (1.1^A and $1.X^A$). The VAT is indicated after the point (for example, 1.Ae), the parent VAT is indicated by a superscript, and a lower case letter is used to designate clones when needed. The 1.A VSG cDNA probe, which contains one *Sal*I site (Fig. 2*d*) also present in each genomic copy of the gene, hybridizes to four *Sal*I fragments in 1.Ae and $1.A^7$ and to three fragments in each of the other clones (Fig. 2*a*). The band at 1.1 kilobases (kb) is composed of co-migrating fragments extending from the internal *Sal*I site to another *Sal*I site present at the same location 5′ to each 1.A gene. The remaining *Sal*I fragments contain the 3′ ends of these genes and allow determination of the number of 1.A genes present in each clone. Like the original VAT 1.A (1.Ae, 15 and 16), the relapse clone $1.A^7$ contains a 1.A ELC. However, no 1.A ELC is present in four other relapse-derived 1.A clones ($1.A^1$, $1.A^3$, $1.A^5$ and $1.A^{11}$). The latter clones, their parent VATs (1.1, 1.3, 1.5 and 1.11) and all other heterologous VATs we have examined (for example, 1.7, $1.X^A$ and 1.1^A) contain only two copies of the 1.A gene. One copy, present on a 13.5-kb *Sal*I fragment, is located in a non-telomeric region and has been cloned in λ_{1059} and extensively mapped (Fig. 4). The other copy is located near a telomere, as evidenced by its size variation among VATs (5–8 kb, Fig. 2*a*) and the presence of a 'universal restriction site' 3′ to the gene (Fig. 4). These data suggest that an ELC is present in some 1.A expressors but absent in others.

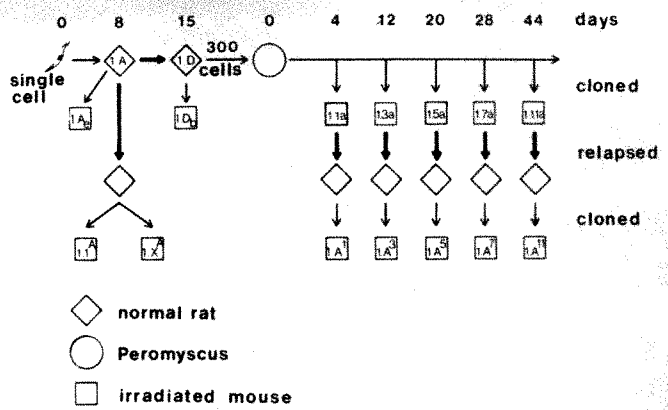


Fig. 1 The IsTAR 1 serodeme[20] was initiated with a single trypanosome cloned from a population of which ~70% of the cells expressed 1.A. The first parasitaemia (day 8) population was designated IsTat 1.A. Three hundred cells of the first relapse population (1.D, day 15) from the same immune competent rat, were injected into a deermouse, *Peromyscus leucopus*. Samples were taken at 4-day intervals, expanded in lethally irradiated mice and then cloned to obtain the VATs shown. Clone 1.Db expresses the same VSG as 1.1a (ref. 15) and thus VAT 1.1 cells were derived from the growth of VAT 1.D cells without an intervening antigenic switch. Approximately 5–500 trypanosomes from each of the six VATs were injected into immune competent rats and the resulting first relapse populations subsequently cloned. The relapse populations from VATs 1.1, 1.3, 1.5, 1.7 and 1.11 were 100% VAT 1.A re-expressors as determined by their reaction with anti-1.A VSG monoclonal antibodies. The relapse population from 1.A yielded two clones expressing 1.1 VSG (from their reaction with anti-1.1 VSG monoclonal antibodies) and one clone expressing a VAT ($1.X^A$) not recognized by any of our VAT specific antisera.

An alternative interpretation of the above data is that the extra bands in 1.Ae and $1.A^7$ are not ELCs but are due to discrete heterogeneity in the length of telomeric sequences flanking a single 1.A gene. Similarly, the presence of ELCs in $1.A^1$, $1.A^3$, $1.A^5$ and $1.A^{11}$ might be concealed by extensive telomeric size heterogeneity, that is, 'ghost ELCs'[21]. To resolve these issues we digested DNA from each clone with *Hin*cII, which cleaves within the VSG 1.A coding region (Fig. 2*d*) and 3′ to each of the 1.A genes (Fig. 4). This allows analysis of the number of 1.A genes independently of telomeric length variation. The 2.0- and 1.3-kb *Hin*cII fragments present in every DNA shown in Fig. 2*b* contain the 3′ ends and the immediate 3′-flanking sequences of the non-telomeric and telomeric 1.A genes, respectively. Densitometric scans (Fig. 2*c*) show a doubling of intensity of the 1.3-kb band compared with the 2.0-kb band, in 1.Ae and $1.A^7$ but not in the other clones. This confirms the presence of a 1.A ELC in these two clones and its absence in the others, and shows that the other telomeric 1.A gene acted as the BC.

The single telomeric 1.A gene in $1.A^1$ is preferentially sensitive to DNase I (Fig. 3), illustrating that the telomeric BC is transcribed in those clones using the NDA mechanism. It is not preferentially DNase I sensitive in non-1.A expressors (P.M., data not shown). In $1.A^7$ (and 1.Ae, not shown), only the ELC is DNase sensitive (Fig. 3*b*), indicating that the duplicated copy and not the telomeric BC is transcribed. Our results therefore show that IsTaR 1 cells can transcriptionally activate the telomeric 1.A VSG gene by either the ELC or NDA mechanisms. While both mechanisms are used for the expression of different VSG gene families in a single serodeme[16,18] and related VSG genes in different serodemes[22], the present report shows that both mechanisms can be used for the expression of a single VSG gene within one serodeme.

Restriction maps of the 1.A VSG genes were constructed by genomic Southern analyses (Fig. 4). Each copy of the 1.A gene has an identical restriction map spanning the coding region from the 5′ *Sal*I site to the 3′ *Acc*I site. Outside this region the

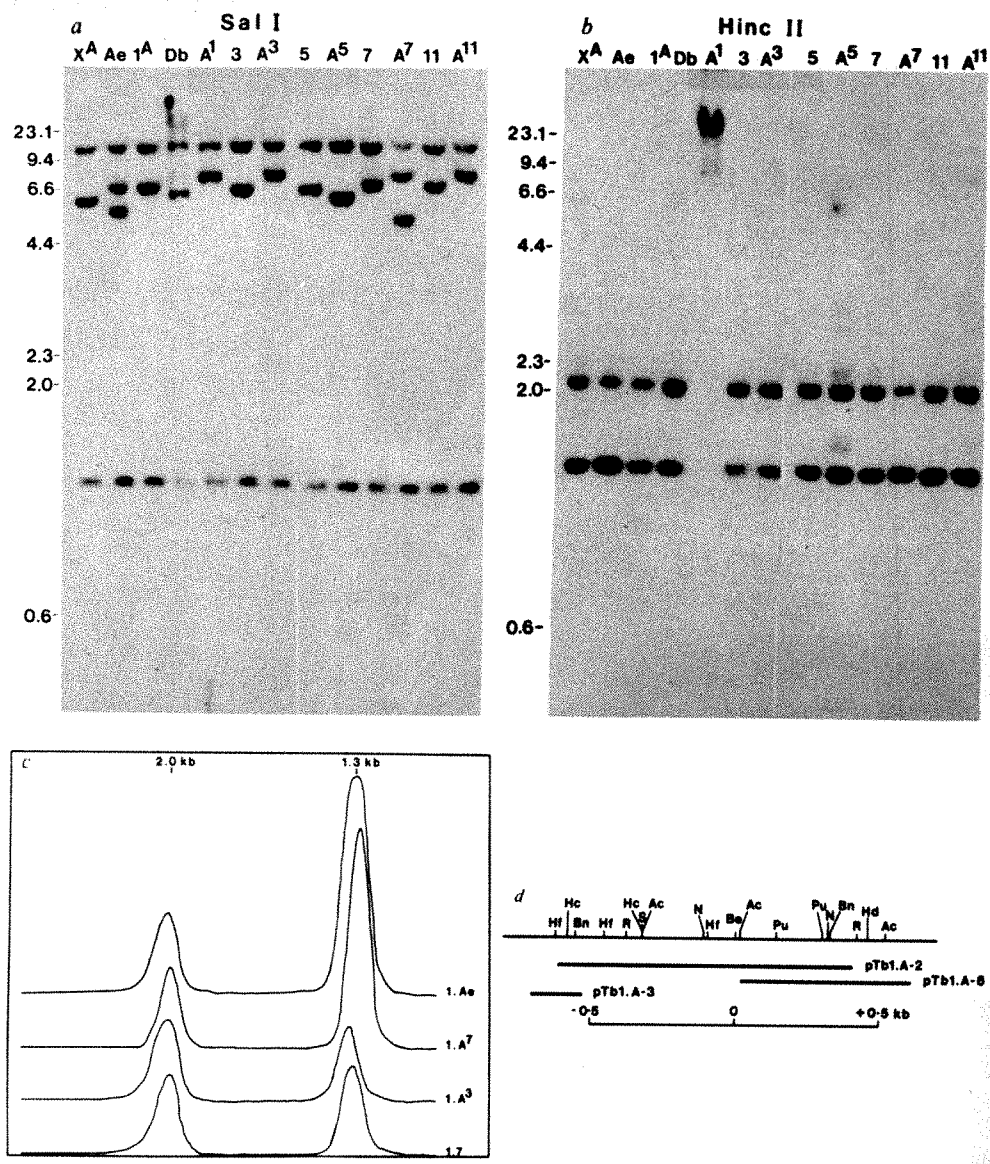


Fig. 2 Southern blot analysis of the 1.A genes in early VATs and cloned relapses. DNA isolated from VATs IsTat 1.X^{A}, 1.Ae, 1.1^{A}, 1.Db, 1.A^{1}, 1.3a, 1.A^{3}, 1.5a, 1.A^{5}, 1.7a, 1.A^{7}, 1.11a and 1.A^{11} (see text for VAT nomenclature) was digested with restriction endonucleases *Sal*I (*a*) or *Hin*cII (*b*) and size-fractionated through 0.6% agarose gels. DNA isolated from VATs 1.Db digested with *Sal*I and 1.A^{1} and 1.A^{5} digested with *Hin*cII did not digest to completion and the small internal and 5′ *Hin*cII fragments ran off the gel in *b*. The gels were blotted bi-directionally onto nitrocellulose filters[35–37], then hybridized with a 1.A cDNA clone (pTb1.A-2) labelled with ^{32}P by nick-translation[38]. Size markers were *Hin*dIII, *Xba*I and *Kpn*I digests of λ DNA and their positions are indicated next to each autoradiogram. *c*, Densitometric scans of autoradiograms from *Hin*cII digests *b* normalized to give the same peak heights for the 2.0-kb band in each of the DNA digests. *d*, Restriction map of clones isolated from a cDNA library prepared from IsTat 1.A (refs 15, 16). Abbreviations used for restriction endonuclease sites are: A, *Ava*I; Ac, *Acc*I; B, *Bam*HI; Be, *Bst*EII; Bg, *Bgl*I; Bl, *Bgl*II; E, *Eco*RI; Hc, *Hin*cII; Hd, *Hin*dIII; Hf, *Hin*fI; P, *Pst*I; Pv, *Pvu*I; Pu, *Pvu*II; R, *Rsa*I; S, *Sal*I; Sp, *Sph*I; Ss, *SSt*I; Xh, *Xho*I.

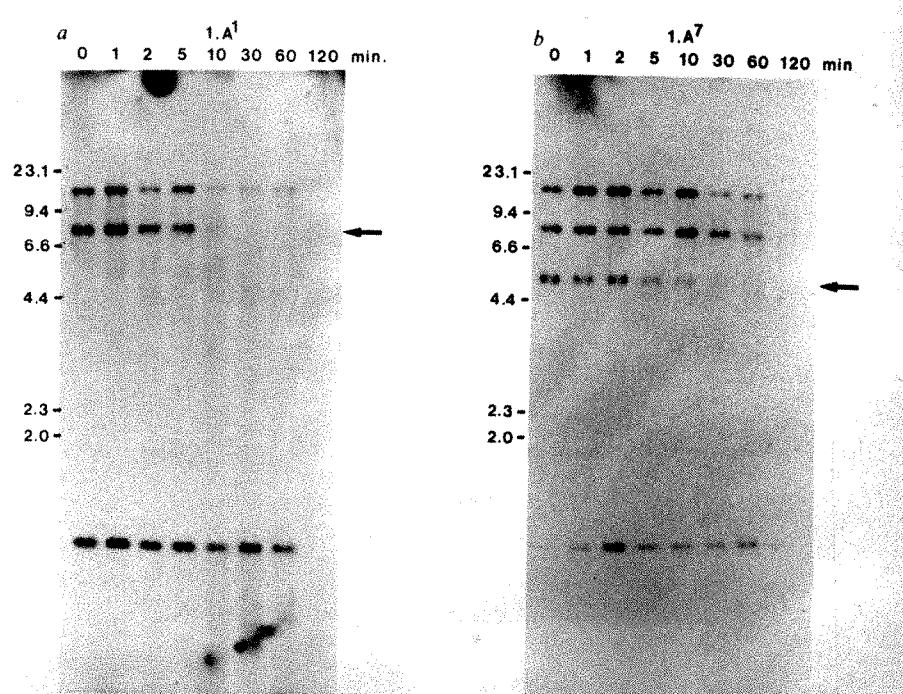


Fig. 3 DNase I sensitivity of IsTat 1.A genes. Frozen trypanosome pellets from 1.A^{1} (*a*) and 1.A^{7} (*b*) were solubilized with Nonidet-P40 and treated with DNase I using the methods of Pays *et al.*[12]. DNase I digested aliquots from the times indicated were deproteinized, digested with *Sal*I, electrophoresed in a 0.7% agarose gel and then blotted onto nitrocellulose. The filter was then hybridized with the 1.A probe (pTb1.A-2). The preferentially digested fragment from the expressed gene copy is indicated by an arrow.

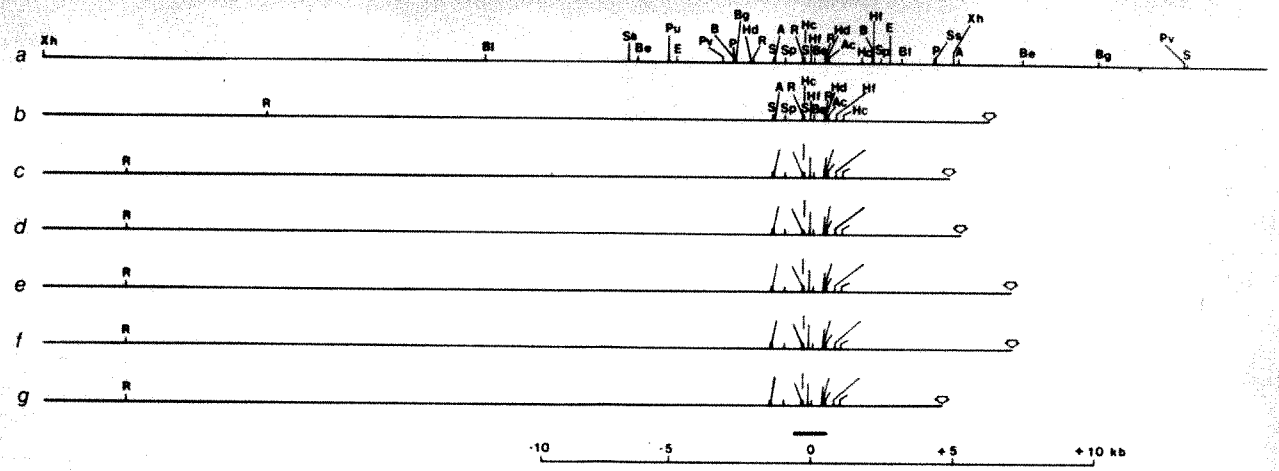


Fig. 4 Restriction maps of IsTat 1.A genes. *a*, The non-telomeric gene in all VATs. *b*, *c*, The ELC and telomeric BC genes, respectively, in IsTat 1.Ae. *d*, *e*, The telomeric gene in IsTat 1.Db and IsTat 1.A[1]. *f*, *g*, The telomeric BC and ELC, respectively, in 1.A[7]. Restriction cleavage sites were determined by Southern hybridization analyses of genomic DNA (see, for example, Fig. 2). Several sites within the coding region have been omitted for clarity (see Fig. 2*d*). The abbreviations for restriction endonucleases used are shown in Fig. 2. The arrow denotes a 'universal restriction site' (*Sal*I, *Ava*I, *Sph*I and *Bst*EII), indicating the telomeric end of a chromosome. The line above the scale indicates the 1.A VSG coding region.

non-telomeric gene (Fig. 4*a*) and telomeric genes (Fig. 4*b–g*) have different restriction maps. Except for the length of the telomere, the maps of the telomeric BC gene (Fig. 4*c–f*) are identical in all clones, whether or not the gene is expressed. Similarly, the map of the 1.A[7] ELC (Fig. 4*g*) is identical to that of the telomeric 1.A[7] BC (Fig. 4*f*). The 1.Ae ELC map, however, differs from these (Fig. 4*b*). The 5′ 'barren' region is 5 kb shorter and the relative order of restriction sites 5′ to the *Rsa*I site distinguishes these telomeric expression sites (P.M., data not shown). The use of more than one expression site has also been seen with other ELCs in the IsTaR 1 serodeme[23] and in the BoTaR serodeme of *Trypanosoma equiperdum*[24], but is in contrast to previous results in *T. brucei*[2,25]. To characterize these expression sites further, blots of restriction digests producing large (up to 50 kb) segments of 1.A 5′ flanking sequence were hybridized with a synthetic probe complementary to a portion of the 35-nucleotide spliced leader found on the 5′ termini of all VSG mRNAs[26,27]. Although the probe hybridized with the large tandem array of directly repeated 1.4-kb units[28,29] previously suggested to be the VSG gene expression site promoter[29], and with orphons[28,30], it did not hybridize to the large fragments containing the 1.A gene. Therefore, none of these spliced leader encoding sequences is within 50 kb of either the expressed or non-expressed 1.A genes.

Both non-telomeric[12,31,32] and telomeric[24,25,33,34] BC genes can be duplicated to form ELCs. The ELCs in both 1.Ae and 1.A[7] are duplicated from the telomeric gene, since all their restriction maps are identical 3′ to the 'barren' region and different from the map of the non-telomeric gene, especially the 3′ *Hin*cII and *Hin*fI sites (Fig. 4). The duplicated segment extends minimally from the 5′ *Sal*I site to the 3′ *Hin*cII site and maximally from the 5′ *Rsa*I site to the end of the chromosome (Fig. 4). In 1.Ae, the 5′ duplication boundary is between the 5′ *Sal*I and *Rsa*I sites. Because the 5′ maps of the ELC and BC genes in 1.A[7] are identical, the boundary in this case may be further upstream. The 3′ duplication boundary in both 1.Ae and 1.A[7] must lie 3′ to the *Hin*cII site. Thus, the ELCs in 1.Ae and 1.A[7] may not result from duplication/transposition of a small 3–5-kb BC 'cassette' spanning the VSG coding region[12,26,27], but rather from a gene conversion initiated 5′ to the coding region and extending to the end of the chromosome. Such a model is consistent with the results presented here and by others[25,34].

We have described a predominant VAT whose VSG gene can be expressed by both the NDA and ELC mechanisms. The fact that the telomeric 1.A gene can be activated by both mechanisms presumably allows its more frequent expression. Furthermore, the similarity between the genomic environments of the basic copy gene and the 'expression site(s)' as shown here and elsewhere[25], may increase the probability of recombination between these two sites. The predominance of 1.A may thus be due to its ability to use alternative mechanisms for VSG gene activation, coupled with a higher frequency of recombination/gene conversion.

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DNA variation and evolution

FERRIS, Sage and Wilson[1] assayed wild-caught and inbred strains of mice (*Mus domesticus*) for variation in mitochondrial DNA (mtDNA) using restriction enzyme digestion. They concluded that the data showed either a common origin of 'old' inbred strains (probably a single female) or contamination from a single strain because all the 'old' inbred strains appeared to share a common mtDNA pattern found rarely (2 mice in 56 tested) in their survey of wild populations.

Their observations were made on samples of single mice from each of 14 inbred laboratory strains (5 'new' and 9 'old') and 15 wild populations; further samples of two and three mice were from two different origins. Thirty-six additional wild mice (origin not stated) were assayed using two restriction enzymes which could discriminate the 'old–inbred' pattern. When all members of a sample of size N are identical for a particular pattern, the 95% confidence intervals on the frequency of that pattern are: for $N = 1$, 0.025–1.00; for $N = 2$, 0.158–1.00; for $N = 3$, 0.292–1.00. Because the size of samples used in these experiments was small, it is possible that substantial levels of variation could remain undetected within the wild populations. Whilst the data may suggest a common origin of the 'old' inbred strains, the lack of data on variation within the wild populations precludes the elimination of the alternative hypothesis of independent origin.

In another paper, Coen, Thoday and Dover[2] based their estimation of rates of 'turnover' in structural variants of the ribosomal gene of *Drosophila melanogaster* on samples of two X chromosomes extracted from 10 pairs of iso-female lines and samples of 'at least two (usually three' X chromosomes from population cages established with a mixture of all iso-female lines. The authors assumed that if a variant was present in at least two sublines, it was probably derived from the founding population. On this basis no novel variants were detected in the paired iso-female lines and the rate of 'turnover' was estimated by assuming that no novel variants had been identified. However, no allowance was made for either the size of the samples or the sizes of the populations from which the samples were extracted. The maximum frequency at which novel variants could exist in all sublines and yet remain undetected ($P = 0.05$) in samples of 2 from 20 sublines is ~0.07. In other words, the iso-female lines could be segregating for novel variants at fairly high frequencies and these would not be detectable because of the small sample sizes.

If these putative variants have arisen since the establishment of the lines, then the maximum rate of their production (m, the mutation or 'turnover' rate) is about 3.74×10^{-4} per generation which is of the same order as values obtained by Coen *et al*[2]. However, these estimates assume that the populations are large and no allowance is made for the finite size of the subline populations. Downes[3] gives estimates of the effective population sizes (N_e) of the OK iso-female lines of between 90 and 400. At these values of N_e and m, the effects of the random sampling of gametes in each generation could have a considerable effect on the fate of new variants because the majority will be lost in the first generation. Consequently, the chances of detecting 'turnover' would be low in such small populations and the measurement of its rate made more difficult by the small sample sizes. Similarly the small samples taken from the cage populations and absence of any information on the effective population sizes restrict the interpretation of the data. The results quoted by Coen *et al.* provide no reasonable *prima facie* evidence of systematic change in the frequency of rDNA structural variants. The estimates of 'turnover' rates are based on the assumption that 'turnover' is occurring, not on a demonstration of its existence.

The reports of both Ferris *et al.*[1] and Coen *et al.*[2] describe the application of the techniques of molecular biology to investigate evolution. The procedures have exposed a range of variation which evolutionary biologists must consider seriously. However, the fascinating interpretations presented in these papers are not strongly supported by the data because alternative hypotheses cannot be eliminated. Acceptance of the conclusions of the two reports must await the availability of more data.

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FERRIS *ET AL.* REPLY—'Concluded' is too strong a term to apply to the various possible explanations we *offered* for the remarkable finding[1] that all nine of the 'old inbred' strains of laboratory mice tested share a type of mtDNA whose frequency is low (0.04) in wild mice (*Mus domesticus*). This finding contrasts sharply with published protein-electrophoretic evidence indicating that the nuclear genes of these laboratory strains are about as different from one another as are randomly picked nuclear alleles in wild mice[1]. It was important not only to bring our finding to the attention of a wide audience but also to consider how to explain this puzzling contrast in light of the evidence that the mode of inheritance of mtDNA molecules differs from that of nuclear genes in being maternal.

Since these nine strains seen from published breeding records to be founded from at least five non-laboratory females, we calculated the probability of picking five mice with the 'old inbred' type at random from the wild. By 'at random from the wild', we meant at random geographically within the entire range occupied by wild members of the species *M. domesticus*. The best estimate of this probability, based on the frequency observed in our wild survey, is $(0.04)^5$, that is, 10^{-7}. This value, which has been confirmed by a more extensive survey[2], was so low that it led us to ask whether: (1) the five founding females were picked nonrandomly; (2) there was selection for the old inbred type; (3) there was frequent, unrecorded, contamination of one laboratory strain by another's mtDNA.

It was our position[1], which our subsequent work on many additional mice confirms[2–4], that none of these possible explanations for the mtDNA uniformly of the old inbred strains can yet be ruled out.

With regard to nonrandom sampling, we pointed to published evidence that most or all of the founding females came from the pet mouse trade. Drift or selection within this population could have brought about a high frequency of the 'old inbred' type of mtDNA. Alternatively, most of the wild mice used to establish or maintain the pet mouse population may have been trapped in a geographically nonrandom way, that is, from a particular region in which the 'old inbred' type happened to be at high frequency. Our initial survey of mtDNA in wild mice was a wide-ranging one, designed to detect all the major mtDNA lineages in *M. domesticus*[1]. We were aware that until a much more extensive survey of mtDNA variability within and between wild populations of *M. domesticus* was conducted, this alternative could not be evaluated. Subsequent survey work[2–4] bringing the total number of wild *domesticus* mtDNAs tested to 145, has confirmed our initial estimate of the frequency of the old inbred type; it has also shown, as we anticipated, that while there is heterogeneity within and between some localities as regards

mtDNA variability in *M. domesticus*, this species displays a rather weak tendency for macrogeographical structuring of that variability[2].

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COEN *ET AL.* REPLY—Genetic turnover by unequal exchange and its evolutionary consequence of molecular drive is well established in the rDNA multigene family of diverse species, since the classic studies of Don F. Brown and colleagues in 1972 (for recent reviews see refs 1–3). To measure the rate of turnover we monitored, over several hundred generations, the stability of rDNA length variants, the expected products of unequal exchange[4,5], in isofemale lines of *D. melanogaster*[6]. Our experimental design was to compare individual X or Y chromosome rDNA variants taken from within each of several pairs of sublines, which had been established by splitting the F_1 progeny of each isofemale line and maintained separately over the prescribed time. By this means the stability of individual chromosome lineages could be assessed. Given that many X and Y chromosome variants from within subline pairs were shown to be identical (that is, no change was observed within these specific chromosome lineages) we estimated the maximal rate of unequal exchange based on the proper statistic that takes into account all independent unchanged lineages across all subline pairs. If the turnover rate were higher than this maximum, we would expect a smaller number of unchanged lineages than was actually observed. We note that Barrett concludes his 'criticism' with a paraphrase of our own summary that in the time course of the experiment "there is no *prima facie* evidence of systematic change in the frequency of rDNA structural variants".

Barrett's appeal for a larger sample size from each subline pair reveals his failure to understand our experimental design and underlines the dangers of applying statistics and population theory in the blind. Our estimated maximal rate is valid irrespective of the number of chromosomes assayed within each lineage and of problems of drift in small populations: it is based only on the number of individual chromosome lineages, each assayed and considered independently. Hence the statistical estimate of our maximal rate would be improved by a wider sampling of more subline pairs, that is, more lineages, rather than more chromosomes within subline pairs.

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100 Hz is not the upper limit of synchronous muscle contraction

SMITH[1] has proposed that 100 Hz is the upper limit of contraction frequency in synchronous muscles—those muscles in which there is a 1:1 correspondence between motor neurone impulses and muscle contractions. In asynchronous muscles, contraction frequency can be much higher than that of the motor neurone impulses. Smith reached his conclusion after demonstrating that whitefly flight muscles with high operating frequency are asynchronous rather than synchronous as was previously thought[2]. 100 Hz may represent an upper limit of wing stroke frequency for synchronous flight muscles, but several synchronous muscles have significantly higher operating frequencies, notably sound-producing muscles in bats, fish, crustacea and insects[3–9].

We have studied cicada tymbal muscles, which contract at high frequency during sound production. These muscles are especially pertinent because a wide range of contraction frequencies is found by examining different species[10] and because both synchronous and asynchronous tymbal muscles occur[11]. Our physiological and fine-structural data show that some synchronous tymbal muscles operate at over 200 Hz (224 Hz in *Psaltoda claripennis*).

In synchronous muscles, high contraction frequencies are made possible by muscle modifications that result in short twitch duration. A comparison of several species of cicadas having synchronous muscles[10] shows a close correlation between twitch duration and contraction frequency (Fig. 1*a*) which extends over the full range of contraction frequencies. However, one species, *Platypleura capitata*, which has asynchronous muscles[11], is vastly different in that it has a very short cycle period during singing but a relatively long twitch duration. This is to be expected as high operating frequencies

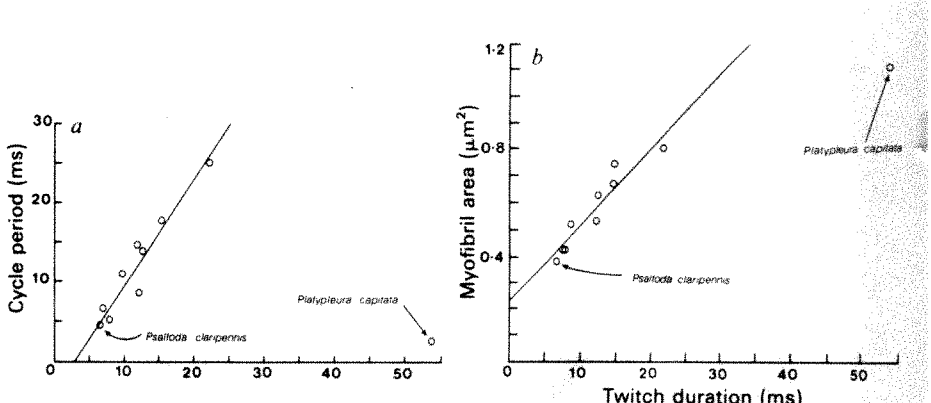


Fig. 1 *a*, The inferred cycle period (=reciprocal of contraction frequency) during the calling song compared with the isometric twitch duration (onset to 50% relaxation, at 30 °C) of the partially isolated tymbal muscles for 10 species of cicada. *b*, Comparison of the myofibril area (measured stereologically from electronmicrographs of transverse sections) with the isometric twitch duration in tymbal muscles for 10 species of cicada. In *a* and *b*, all the species have synchronous tymbal muscles except for *Platypleura capitata*, which is asynchronous. The lines are least-squares regression lines for the data, excluding *P. capitata* ($r = 0.95$ in *a* and *b*). In *a*, the unlabelled points represent the following species, in order of increasing twitch duration: *Psaltoda harrisii, Psaltoda argentata, Magicicada cassini, Magicicada septendecim, Cyclochila australasiae, Abricta curvicosta, Chlorocysta viridis* and *Cystosoma saundersii*. Details of these species are presented elsewhere[10,11]. Two species (*Arunta perulata* and *Tamasa tristigma*), in which there is some doubt about the cycle period during calling, are not included in this graph. In *b*, the unlabelled points represent the following species, in order of increasing twitch duration: *P. harrisii, P. argentata, T. tristigma, C. australasiae, A. curvicosta, A. perulata, C. viridis* and *C. saundersii*. Details of tymbal muscle fine structure for these species will be published elsewhere (R.K.J. and D.Y., in preparation). Data for *P. capitata* are given in ref. 11. *M. cassini* and *M. septendecim* were not included in the study of fine structure.

in asynchronous muscles are achieved by elaboration of stretch activation/release inactivation in muscles that basically have twitches of long duration[12,13].

Synchronous and asynchronous muscles can be distinguished by their fine structure, as Smith[1] points out; the former possess an extensive sarcoplasmic reticulum and T-system and tend to have small myofibrils, whereas the latter tend to have a very reduced sarcoplasmic reticulum and large myofibrils. In cicadas, twitch duration in synchronous tymbal muscles is inversely related to the quantity of sarcoplasmic reticulum within each muscle fibre (R.K.J. and D.Y., in preparation). The faster tymbal muscles also have smaller myofibrils, with myofibril cross-sectional area being the best morphological predictor of twitch duration (Fig. 1*b*). The asynchronous muscle of *P. capitata* is quite different from fast, synchronous muscle (for example, that of *P. claripennis*) in having a large fibril area and in being sparsely supplied with sarcoplasmic reticulum[11].

Thus, synchronous tymbal muscles constitute a graded series, throughout which contraction kinetics and fine structure are closely correlated with the normal operating frequency. We have demonstrated that contraction frequencies of over 200 Hz are achieved within the synchronous mode of operation. Clearly, 100 Hz is not the upper limit of contraction frequency in synchronous muscles.

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Encephalization in *Proconsul africanus*

WALKER *et al.*[1] recently suggested that the Miocene hominoid *Proconsul africanus* was more encephalized than extant monkeys of similar body size, and they suspected that it may resemble pongids in relative brain size. As an index of encephalization they used an encephalization quotient (EQ) based on a particular allometric equation[2]. Here I re-examine the degree of encephalization of *P. africanus* by applying two additional, but different, indices of encephalization traditionally used in brain–body size studies: one based on the constant of cephalization (CC)[3–5], the other on the extra neurone index (N_c)[6]. Both approaches yield the same result, which is in conflict with that of Walker *et al.* According to these methods, the degree of encephalization of *P. africanus* is within the range of extant monkeys and substantially below that of the apes.

Table 1 Encephalization indices in *Proconsul africanus* and extant apes and monkeys

	EQ	CC	N_c ($\times 10^9$ neurones)
Proconsul africanus	48.8	19.6	1.9
Pan troglodytes	41.1	35.6	3.6
Pongo pygmaeus	31.6	30.1	3.3
Gorilla gorilla	17.2	31.2	3.5
Cercopithecoidea	22.9–82.0	8.0–20.2	0.9–2.1

EQ = brain weight (g)/0.0991 (body weight, g)$^{0.76237}$; CC = brain weight (g)/(body weight, g)$^{0.23}$; for calculation of N_c, see ref. 6.

The equation used by Walker *et al.* provides an excellent fit for brain–body size relationships in high-level taxa such as the class Mammalia (mouse–elephant curve) or the order Primates (mouse lemur–gorilla curve). Because of its high exponent of allometry (0.76), however, it tends to overestimate the degree of encephalization for small-sized species while underestimating encephalization in larger species, when comparisons are made at a lower level of the hierarchy. This is the case because among closely related species or genera, brain weight scales at a much lower power of body weight with values of the exponent of allometry characteristically falling between 0.2 and 0.3 (refs 3, 4, 7, 8). In the study by Walker *et al.* this phenomenon leads to obviously erroneous results such as gorillas being less encephalized than the least encephalized monkey and the range for monkeys surpassing that of the apes at the upper end by a considerable margin (Table 1). Being aware of the strong limitations of their method, Walker *et al.* resort to a comparison of *P. africanus* with similar sized monkeys. While this alleviates the problem somewhat, the fact that the comparison of EQs is based on an equation fitting high-level taxa still leaves a major source of error.

Using the same brain–body size data but an equation based on an exponent of allometry (0.23) consistent with brain–body size relationships among low-level taxa, yields not only different but much more consistent results (Table 1). The CC values for the apes are closely grouped together and substantially above the range for monkeys. *P. africanus* lies towards the upper end of the monkey range. For *P. africanus* to be at the bottom of the range of apes (CC = 30) would require a cranial capacity of 255 cm^3, almost 90 cm^3 larger than the actual estimate, at a body weight estimate of 11 kg. Use of the extra neurone index yields the same results (Table 1): apes fall closely together and are clearly above the monkey range with *P. africanus* in the upper end of the latter.

Thus, while *P. africanus* may share several derived traits with extant apes, relative brain size does not seem to be one of them.

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SMITH AND WALKER REPLY—Leutenegger's discussion and data are a useful addition to the interpretation of relative brain size in *Proconsul africanus*, but careful evaluation of his data confirms our conclusion that *P. africanus* shows a tendency towards the increased encephalization that characterizes apes in comparison with monkeys. The indices used by Leutenegger do not eliminate the confounding effect of size that he correctly noted exists for the values that we reported when using a slope of 0.76 to calculate encephalization quotients.

While our measurement of relative brain size involved an inverse bias—animals with larger body weights having lower EQs—both indices used by Leutenegger show the opposite bias—larger animals having larger values. He reports Hemmer's[1] values for the constant of cephalization (CC) as being in the range 8.0–20.2 for Cercopithecoidea. The value of 8.0 is for the smallest cercopithecoid, *Miopithecus talapoin*, and the largest

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values are for several of the largest species in the group, all from the genus *Papio*. With the extra neurone index (N_e), Jerison[2] reported that elephants have a value twice that of humans and porpoises have about the same values as we do. At the other end of the scale, the small South American squirrel monkey, *Saimiri sciureus*, was grouped with lemurs and marmosets rather than with more closely related, but larger, New World monkeys. Jerison himself noted that "If we demand that the values of N_e correspond to an ordering in terms of behavioural capacities we must assume either that the assumptions used in determining N_e are insufficient or that we grossly underestimate the behavioural capacities of the elephant and porpoise. I would guess that both types of error occur, but I would prefer, for the present, to emphasize the second."

If, as these results suggest, there is a tendency for CC and N_e to be underestimated for small species within a taxon, our suspicions of increased encephalization in *P. africanus* remain plausible. For both CC and N_e, the values for *P. africanus* listed by Leutenegger are close to the highest values for all monkeys and the monkeys with the higher values are also considerably larger than *P. africanus* in body size. *P. africanus* has lower values than the extant great apes, but also has a much smaller body size than these.

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RECENTLY Toh *et al.*[1] reported the existence of amino acid sequence similarity between the reverse transcriptase of certain retroviruses and the DNA polymerases of cauliflower mosaic virus (CaMV) as well as a region of hepatitis B virus (HBV) that probably encodes a DNA polymerase. For completeness, we draw attention to other regions of protein similarity between retroviruses (human T-cell leukaemia virus, HTLV; Moloney murine leukaemia virus, Mo-MuLV; and Rous sarcoma virus, RSV), HBV and CaMV that were not reported by Toh *et al.*

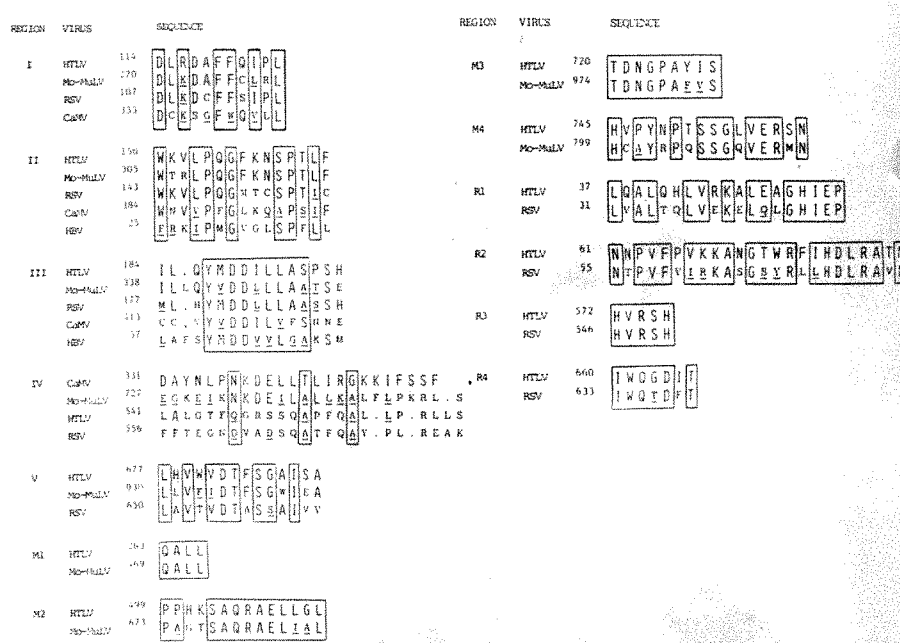
The sequences are presented in Table 1 and the relative positions of the similar regions indicated in Fig. 1. The common set of amino acids discussed by Toh *et al.* is conserved in the amino-terminal region of all retrovirus reverse transcriptases studied (regions I–III). These common sequences are located near the amino-terminus of the HBV protein and the centre of the CaMV polymerase. Another region of similarity, IV, not discussed by Toh *et al.*, is also found in all retroviruses and is present in the CaMV protein. Region V, reported by Toh *et al.*, to be present in Mo-MuLV and RSV, is also found on HTLV. We also draw attention to sets of amino acids that are conserved with respect to sequence and relative position between the reverse transcriptases of HTLV and Mo-MuLV (M1–M4) and HTLV and RSV (R1–R4).

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Fig. 1 Alignment of the polymerase gene products among five different viruses, depicting the regions shown in Table 1.

Table 1 Alignment of the similar regions in polymerase gene products among different viruses



Moloney murine leukaemia virus, Mo-MuLV[2]; human T-cell leukaemia virus, HTLV[3]; Rous sarcoma virus, RSV[4]; cauliflower mosaic virus, CaMV[5]; and hepatitis B virus, HBV[6]. Common amino acids are boxed and conservative substitutions with respect to HTLV are underlined. For each sequence, the first amino acid is numbered.

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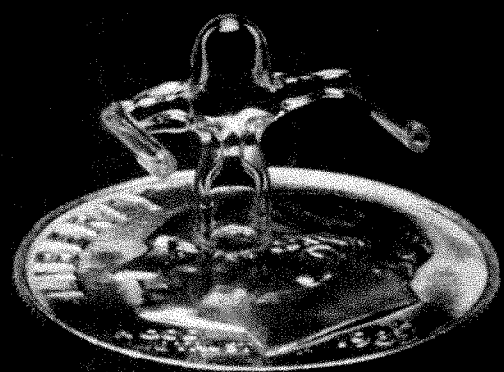
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Primatology by numbers

Bernard Wood

The Order of Man: A Biomathematical Anatomy of the Primates. By Charles Oxnard. *Yale University Press: 1984. Pp.366. $30, £20.*

THE manner of acquiring knowledge about ourselves and our closest relatives, the primates, perceptibly changes every few years, so that the past two or three decades have witnessed the virtual transformation of primatology. Prior to this, apart from observations of captive animals and a few pioneering field studies, primatology was all but confined to classical comparative anatomical and palaeontological research. Primatology, or more accurately primate evolutionary biology, has now matured into a discipline which embraces studies as disparate as molecular evolution, ethology and comparative neurobiology.

Nonetheless, investigations of the form of the skeleton, and comparisons of homologous skeletal parts, still play a prominent part in primate studies. The advent of computers, along with statistical techniques which allow several variables to be considered simultaneously, have progressively added to the power and range of these so-called morphometric methods, and this book is the most recent of a trilogy* by Charles Oxnard which describes the basis, application, evolution and impact of the newer morphometric techniques. *The Order of Man* is particularly interesting because, more than either of the other books, it attempts to set these studies within the broader context of primatology.

Morphometry is the reduction of a complex form, usually a bone, to numbers. Present-day techniques are sophisticated in three respects. The first is the ingenuity of the ways in which shape and size are recorded: simple linear measurements are being superseded by three-dimensional coordinate and orthogonal analysis, as well as by optical techniques. The second is the application of morphometry to the internal structure of bone, thus increasing the comparative information which can be gleaned from skeletal material. Finally, there is the statistical treatment of the data. Multivariate techniques have been refined and elaborated so that the elements of a complex three-dimensional shape can be visualized and more easily compared with other homologous patterns.

In the United Kingdom, it was the Birmingham School of anatomists, led and inspired by the now Lord Zuckerman, who, in the post-War period, brought bivariate, and then multivariate statistics to the study of the skeletons of living and fossil primates. Claims for the recognition of new and different sorts of hominids were often, and sometimes correctly, based on morphological features; but, with some honourable exceptions, little attempt was made to support these decisions with quantitative data. To those unfamiliar with the morphology of such material it was easy, but unfair, to interpret all this work as unsound. Nonetheless, even when data could be assessed metrically, Zuckerman and his statistical collaborators saw the flaw in methods which implied that each measurement provided a separate "proof" of difference or affinity. They pioneered the introduction and use of simple statistical tests, and later used multivariate methods which were able to identify, and allow for, correlation and covariance between measurements. This work was continued by E.H. Ashton and Oxnard, who represent the first and second generations of the Birmingham School. Though much of their work has been collaborative, Oxnard has emerged as the spokesman, and indeed the doyen, of primate morphometry. So this book doubles as a statement of personal scientific philosophy, as well as being a prospectus for the morphometric cause.

Morphometry has two main applications in primatology — the analysis of form in terms of, first, function and, second, affinity. As I understand it, the gist of the first application, as it has been applied to locomotion, is the following. Individual primate taxa show a range of locomotor and positional behaviours. Thus, the skeleton has to tolerate a range of imposed stresses and strains. These methods seek to find out whether some activities — leaping and hanging, for example — impose such constraints on skeletal variation (or vice versa) that the activity can be recognized in the bones. The second application, the essentially phenetic comparison of form, has found most use in the study of fossils, particularly fossil hominids. All fossils, like living individuals, are unique, but the problem is how to assess the significance of the differences between them. If they are greater than the variation in analogous modern taxa, or are qualitatively different from those seen in intraspecific variation, then taxonomic distinction is warranted. Morphometry provides the means to make these comparisons and assessments.

Most of *The Order of Man* is taken up with describing a series of case studies in which morphometry has been applied to various regions of the skeleton. The detractors of multivariate methods will claim that what these studies have revealed is evident to an experienced anatomist, but this is altogether too glib an assessment. By untangling the web of intercorrelation, such methods reveal patterns of association otherwise concealed from conventional analysis, and some studies, particularly those on the scapula and the pelvis, have provided important functional insights. However, perhaps more interesting than the results themselves is the way that Oxnard regards them. He apparently no longer sees these techniques as part of the armamentarium of the primatologist, but instead regards morphometry as one of three, seemingly discrete, approaches which can be used to determine relationships among primates (the other two being classical morphology and molecular evidence). Oxnard appears to see these technique-based divisions as somehow being in competition, and he points to the concordance of patterns shown by the molecular and morphometric techniques as evidence of the efficacy of the latter.

Apart from the dubious logic of such tests, the examples of concordance which are quoted are not as reliable as is implied in the text. For example the apparent agreement between morphometric and molecular evidence, that *Tarsius* is the sister group of the anthropoids, is contrasted with the conclusions of classical studies which class it as a prosimian. However, the molecular evidence for *Tarsius* is at best equivocal, and there is cogent evidence from classical morphology to suggest that *Tarsius* should be included, with the anthropoidea, in the Haplorhini. These details apart, I am dismayed to see a holistic approach to primate studies abandoned in favour of a compartmentalized one. It neglects other important lines of evidence, behaviour for example, and it implies that the three broad approaches provide the same type of information. Although some multivariate techniques take account of within-group correlation, the raw material of morphometrics is phenetic data. While some — perhaps even most — of this data reflects patristic affinity, part of it is likely to reflect parallelism and convergence, and thus confound attempts to determine "true" relationships. This philosophical shift in the way Oxnard views the evidence of comparative morphometrics, towards regarding it as suitable grist for the primate systematic mill, is thus a major change. It is also one which runs counter to the emphasis in recent years, exemplified by cladistic methods, which stresses the importance of analysing the total form of an animal or a bone, to distinguish between characters which are shared with a wide range of taxa, and those which are unique to a set of taxa, or a single taxon.

The last part of the book concentrates almost exclusively on assessing affinities,

**Form and Pattern in Human Evolution* (1973); *Uniqueness and Diversity in Human Evolution: Morphometric Studies of Australopithecines* (1975). Both published by The University of Chicago Press.

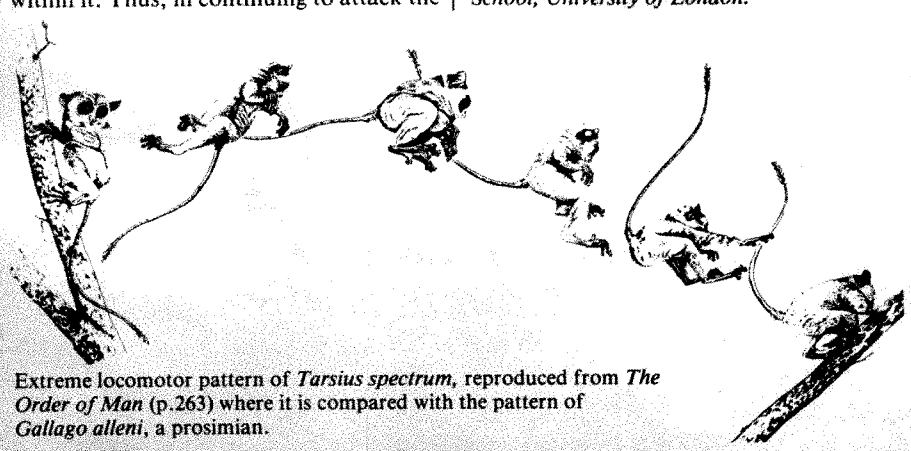
particularly those of hominid fossils. Along with many other workers, Oxnard and his colleagues have used morphometric and other evidence to demonstrate convincingly that the australopithecines are different from both modern man and the extant apes; in short, that they are unique. This generalization has been accepted by most hominid palaeontologists, even though some specimens such as the foot (OH 8) from Olduvai are still the object of the palaeontological equivalent of hand-to-hand fighting. Oxnard is right to claim credit for contributing to this conclusion. But he is wrong to suggest that it was morphometric evidence alone which brought about the change.

There is, however, less agreement about the significance of this phenetic uniqueness. While Oxnard and his colleagues regard it as debarring both the robust and gracile australopithecines from an ancestral relationship to *Homo*, others see one, or both, of the gracile australopithecine taxa as potential ancestors of either the *Homo* or robust australopithecine clades, or perhaps both. The former position would be vindicated if a more suitable *Homo* ancestor were to be found which was either synchronic with, or older than, the australopithecines. Oxnard makes this prediction explicit in the book, so it is particularly puzzling that he includes a *nota bene* section which draws attention to the generally "ape-like" morphology of *Australopithecus afarensis*. If anything, current interpretations of this earlier form of gracile australopithecine tend to emphasize the relatively *Homo*-like features of *A. africanus*, and it is by no means certain that, despite its "morphometric" uniqueness, it can be excluded as a common ancestor of both *Homo* and the robust australopithecines. It is simply not true, nor has it been for a decade or more, to say that patterns of human evolution in the literature "all conform to the notion of a single lineage". Since the abandonment of the "single-species" hypothesis most, if not all, hominid phylogenetic trees have incorporated a separate robust australopithecine lineage, and the recent schemes of Johanson, White and Kimbel incorporate the gracile australopithecines within it. Thus, in continuing to attack the unilinear evolutionary model, Oxnard spends most of the concluding chapter delivering a *coup de grace* to an opponent that has long since left the field. Oxnard is not familiar with the fossil evidence, and it shows throughout this section.

The design of the book and style of the text deserve comment, for they reflect Charles Oxnard's laudable desire to innovate. The layout is pleasing to the eye, but at times infuriating for the reader. Many figures are three or four pages away from the relevant text, and one is eight pages adrift. Despite the desire expressed in the preface that *The Order of Man* should be accessible to the beginner and the lay-person, and the inclusion of an abstract and a summary for each chapter, this is clearly not a book for the non-specialist. Nobody is better at explaining these difficult techniques than Oxnard, and indeed the early chapters discuss some general methodological problems with commendable conciseness and clarity. But most of the book is made up of highly technical diagrams, and the text takes for granted a considerable grasp of primate taxonomy and of systematics on the part of the reader.

The Order of Man is not merely a compilation of the published work of the descendants of the Birmingham School, yet neither is it an objective or comprehensive review of the contribution of morphometrics to primate studies. There is a dearth of self-criticism, and perhaps a surfeit of self-congratulation. It is as if the author were so impatient of the assessments of others, or so exasperated by the lukewarm response of his peers to published analyses, that he decided to act as his own judge and jury. Oxnard and his colleagues have offered much to current debates in primatology, but this text makes it no easier to understand where they are bound, apart from the prospect of mega-computations based on data from both limbs and trunk combined. I fancy that in the decades ahead this unusual book will be of as much interest to historians of science as it will to primatologists. □

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Extreme locomotor pattern of *Tarsius spectrum*, reproduced from *The Order of Man* (p.263) where it is compared with the pattern of *Gallago alleni*, a prosimian.

Vandalized space

Ian G. Halliday

Quarks, Gluons and Lattices.
By M. Creutz.
Cambridge University Press: 1983.
Pp.169. £17.50, $34.50.

RELATIVISTIC quantum field theory poses some of the hardest and most intriguing problems known to theoretical physicists. On the one hand the divergent perturbation series of quantum electrodynamics correctly predicts the magnetic moment of the electron to eleven significant places. On the other hand the experimentalists insist that protons are made of quarks, which refuse to behave as naive analogues of the proton and neutron in the nucleus or of the electron and proton in the hydrogen atom. No matter how hard one bangs protons together no quarks come out. Yet starting from a Hamiltonian written in terms of quark and spin coloured gluon fields, any application of perturbation theory would give free quarks.

So, theoretical physicists have been forced to alternative non-perturbative approximation schemes. The most popular is, at first sight, the most unlikely. Space-time is vandalized and reduced to a discrete, finite volume lattice. This kills all the sacred cows of translation invariance, Lorentz invariance and rotation invariance. We are off to a bad start. However, the system now consists of a finite number of degrees of freedom. Thus it is amenable to direct numerical simulation. Also, it is now very like a solid-state problem where much intuition has been accumulated. For example, near a second-order phase transition the correlation length or inverse mass goes to infinity and details of the lattice are washed out. This is the route to recovering Lorentz invariance! The limit must be taken in parameter space to a point where the correlation length becomes infinite in lattice spacings. This is equivalent to the lattice spacing tending to zero as required to recover the original field theory.

The purpose of Mike Creutz's elegant book is to provide a gentle introduction to the basic results in lattice field theory. It succeeds admirably. Without drowning the reader in detail, sufficient background is given to make the book very readable and provide an easy route to the research literature. Enough examples of calculations are given to develop real confidence in manipulating gauge groups, group integrals, duality and all the other tools of the trade. I recommend the book in the strongest terms to all interested in the fundamental structure of nature and the surprises quantum field theory has in store.

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Focus on fleas

Harry Hoogstraal

The Rothschild Collection of Fleas: The Ceratophyllidae.
By Robert Traub, Miriam Rothschild and John Haddow.
Published privately (distributed by Academic Press): 1983. Pp.494. £60, $99.

WE have here a contradiction to the statement: "No great and enduring volume can ever be written on the fleas, though many there be who have tried it" (H. Melville in *Moby Dick*, Chapter 104). Preceded by five volumes of the *Illustrated Catalogue of Fleas (Siphonaptera) in the British Museum (Natural History)*, this review of the Ceratophyllidae has been particularly difficult and time-consuming to prepare. Since 1936, when the last critical review of the family was published, the number of component species has increased from 71 to about 470 in 65 genera and subgenera, or about 20% of all known fleas.

As in the previous volumes, the specimens in the Rothschild and British Museum collections are listed. However, because of the sheer number of ceratophyllid species, the format of this volume differs from that of the first five. The keys (by F.G.A.M. Smit) to genera and subgenera (seven genera and eight subgenera are described as new) are preceded by a detailed account of morphology and a table of comparative morphology of the closely related family Leptopsyllidae, and are illustrated by 205 line-drawings and 90 photomicrographs (by J. Navarro and R. Traub) of definitive structures of each genus.

The sections on zoogeography and distribution, and the notes on hosts, include comments on the ecology of each ceratophyllid species. A distribution map accompanies the introductory remarks for each genus. The 151 full-page maps show collecting localities of each species on a background of the distribution of the chief host — a formidable task for much of the world, when dealing with so many different flea and host species from so many remote localities.

The review of hosts of ceratophyllids is a classic exposition of host–parasite relationships, special reference being made to the historical distribution of the chief hosts (especially the more-recently evolved Sciuridae, Cricetidae, Geomyidae; and certain birds). It appears to me that the nesting types of many important avian hosts (woodpeckers, swallows, weaver birds, buntings and so on) of ceratophyllids may have a significant role in their infestations by these fleas. The reasoning ("negative evidence") for the dearth of ceratophyllids on Muridae, other rodents and mammals of other orders is as important for biologists to understand as the reasons advanced for why ceratophyllids parasitize their chief mammalian hosts.

This section leads on to an equally valuable account of the evolution of the Ceratophyllidae from the standpoints of historical biogeography and host-relationships, morphology, taxonomy, phylogeny, physiology, behaviour and geological age. The conclusions are that these fleas, like their major and aboriginal hosts (Sciuridae), arose in the Nearctic Region about 30–40 million years ago (early Oligocene) and that they are also the most recently evolved and highly developed group of Siphonaptera.

In the chapter on medical importance, Traub stresses that ceratophyllids do not have a large role in the epidemiology of plague and murine typhus but are clearly important as vectors of zoonotic agents among small mammals, primarily rodents, which are the ultimate source of human disease. The introductory remarks on factors affecting epidemiology illustrate Traub's broad experience in the area of arthropod-borne diseases. Ceratophyllids are discussed in relation to plague, tularemia, other bacterial infections, rickettsiae, viruses, and other infections and medical aspects.

Three appendices complete the book, the first listing species described since 1975, the second dealing with the orders of hosts (and the number of families, genera and species in each) and the number of ceratophyllids (genera/subgenera) and other flea families associated with these host orders and families. Finally, Appendix 3 is an alphabetical list of host genera together with the major ceratophyllid species infesting each.

In the preface, the authors state that their aim was to provide a sound base for future work on this family of parasites and to assist the medical entomologist in the task of identifying his specimens. They have amply fulfilled their mission, and have contributed much solid data and conceptual wisdom to the biomedical sciences. □

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Policy for science

Harvey Brooks

Science in Government: A Review of the Principles and Practice of Science Policy.
By J. Ronayne.
Edward Arnold: 1984. Pp.250.
Pbk £9.95, $19.95.

FOR the reader familiar with recent writings on science policy, there are few surprises or new insights in this book. However, it is an excellent and comprehensive review of a diverse and scattered literature — I know of no other book where this material has been brought together in such an organized and coherent way.

Ronayne interprets the term "science policy" as "policy for science"; there is little discussion of the use of science to inform political decisions about non-scientific issues — what is usually termed "science for policy". He deals with how national governments are or should be organized to support scientific research and development; the theory and practice of determining priorities among areas of research; the pervasive tensions between the claims of the scientific community for autonomy and the demands of society or the political system for accountability; the rationales for government intervention or non-intervention in the industrial innovation system; the role of science policy in fostering industrial innovation and economic growth, and the contribution of basic research to the economy; and the relative merits of centralized and decentralized (or pluralistic) approaches to science policy.

In the accountability versus autonomy debate, Ronayne concludes that society should determine the broad goals of research and areas of emphasis, while leaving questions of tactics and execution to the scientific community. On the question of scientific priorities, he is sceptical of the value of formalized analytical techniques of assessment, except possibly as an indirect stimulus to more systematic thought about opportunities and needs. He quotes with approval a University of Sussex dictum that "cost-benefit studies should not be undertaken...when the results may be taken seriously". Finally, on centralism versus pluralism, he seems to recommend a middle course, somewhat biased towards a semi-centralized position, in which "countries employ their scientists and technologists in a concerted manner to attack the problems that emerged in the new economic context".

Most of what is available in the literature consists either of advocacy or of multi-authored collections in which no attempt is made to contrast and analyse various perspectives. In addition to providing a comparative view, the book contains excellent descriptions of the government organization for science sponsorship and science policy in most of the major industrialized countries. It should thus provide a valuable textbook for an introductory course in science policy, and it deserves a wide audience. One unique feature is the first chapter, which presents a condensed history of the evolution of science policy from the beginnings of modern science in the seventeenth century; I found this the most original aspect of the book. □

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Recombinant DNA: A Short Course. By J.D. WATSON, J. TOOZE and D.T. KURTZ. *W.H. Freeman: 1983. Pp.260. Hbk ISBN 0-7167-1483-3; pbk ISBN 0-7167-1484-1. Hbk £24.95; pbk £14.95.*

Rhythms in Biology and Other Fields of Applications: Deterministic and Stochastic Approaches. Lecture Notes in Biomathematics, Vol.49. Proceedings of a Meeting held September 1981, France. M. COSNARD, J. DEMONGEOT and A. Le BRETON (eds). *Springer-Verlag: 1983. Pp.400. Pbk ISBN 3-540-12302-4/0-387-12302-4. DM 56, $22.30.*

Selected Topics in Preventive Cardiology. Ettore Majorana International Science Series, Vol.12. A. RAINERI and J.J. KELLERMANN (eds). *Plenum: 1983. Pp.275. ISBN 0-306-41375-2. $45.*

Sexually Transmitted Diseases in Homosexual Men: Diagnosis, Treatment and Research. D.G. OSTROW, T.A. SANDHOLZER and Y.M. FELMAN (eds). *Plenum: 1983. Pp.272. ISBN 0-306-41337-X. $35.*

NATURE CLASSIFIED

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MONASH UNIVERSITY
Melbourne, Australia
CHAIR OF EARTH SCIENCES

Applications are invited for the position of Professor and Chairman of the Department of Earth Sciences.

Earth Sciences is an active department which has rapidly gained an established national and international reputation for the high quality of its graduates and research. With nine academic staff and eight technical staff, it covers a wide range of interests, including structural geology, igneous, metamorphic and ore petrology/geochemistry, sedimentology, marine geology, palaeoclimatology, palaeontology, and regional geology and tectonics. A teaching and research program in geophysics begins in 1985 with the appointment of two academic staff. The postgraduate body includes six post-doctoral research workers and twenty-five PhD and MSc students.

The Department is well equipped for experimental and analytical studies and has strong field-based interests. It enjoys good relationships with industry and is involved in joint research projects. The Department has an excellent record of attracting research funds from Australian and overseas grant bodies.

An innovative earth scientist with a strong research reputation is sought to administer the Department and guide future development. The successful applicant must have outstanding leadership and administrative qualities, and the ability to interact positively with academics and industry. Applicants' interests should strengthen and/or complement existing teaching and research in Department. It is hoped that the new Chairman will take up duty in early 1985.

Enquiries about the Department may be directed to Dr Ian Nicholls, Deputy Chairman.

Salary: A$51,006 p.a. Superannuation, travel and removal allowance, and temporary housing assistance.

Information on application procedure and further particulars may be obtained from the Registrar, Monash University, Clayton, Victoria 3168, Australia, or the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF.

Applications should reach the Registrar not later than 30 June 1984. Council reserves the right to make no appointment or to appoint by invitation at any stage.

An Equal Opportunity Employer.
(2023)A

UNIVERSITY OF BRISTOL
DEPARTMENT OF BOTANY
ICI POST-DOCTORAL FELLOW

Applicants should have recently completed their PhD in bacteriology and/or mycology. A background knowledge of plant pathology and agriculture would be most desirable, and a clean car driving licence is essential. The project will run for two years, extendable for one further year, and will (1) develop biological control agents against soil-borne diseases and, (2) look for other agriculturally useful bacteria. The starting date is 1st July or as soon thereafter as possible.

Applications, with two copies of curriculum vitae and the names of two referees, should be sent to Dr R Campbell, Department of Botany, University of Bristol, Woodland Road, Bristol BS8 1UG, England, by 31st May, 1984.

RESEARCH TECHNICIAN

Applications are also invited for the post of techician to work on the above project. Details and application procedure as above. (2051)A

QUEEN ELIZABETH COLLEGE
Kensington
(University of London)
BIOCHEMISTRY DEPARTMENT
CYTOGENETICIST

required for a leukaemia Research Fund supported project mapping human genes associated with chromosomal changes in leukaemias. Experience in cytogenetics is essential and an MSc or PhD are desirable. The post will provide the opportunity for a skilled cytogeneticist to learn some techniques of molecular genetics and recombinant DNA. The post is tenable for three years and is available immediately. The salary will be up to £8530 pa, depending on age, qualifications and experience, plus £1186 pa London Allowance.

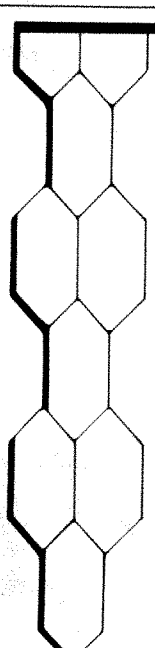
Applications with curriculum vitae and the names of two academic referees should be sent to Mrs. J. Staight, Assistant College Secretary, Queen Elizabeth College, Campden Hill Road, Kensington, London W8 7AH. Further details may be obtained from Dr. Susan Malcolm by telephoning 01-937 5411.

Closing date: 1st June 1984.
(2031)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
"NEW BLOOD" LECTURER IN MEDICAL VIROLOGY

Applications are invited from Science (PhD) or Medical graduates, aged less than 35 years, with a proven record in virological research. The Head of the Laboratory is Dr P D Griffiths (extension 3210) and current interests are herpes viruses, rapid diagnosis, immunosuppressed patients and cell-mediated immunity.

Medically qualified applicants: salary on scale £9,490 — £16,440 plus £807 London Weighting Allowance (with appropriate honorary clinical status at the Royal Free Hospital.) Non-medically qualified applicants: salary on scale £7,190 — £11,615 (under review) plus £1,186 London Weighting Allowance. Superannuation benefits. Eight copies of curriculum vitae and names of two referees to be sent by 8th June 1984 to the School Office, RFHSM, Rowland Hill Street, London NW3 2PF, (01-794 0500 ext 4262) from which further details may be obtained. (2018)A



Chemical Thermodynamicist

BP Research Centre is looking for a Chemical Thermodynamicist to join its team in Petroleum Engineering Branch investigating various aspects of the phase behaviour of hydrocarbon fluids. The post offered is for the study of transport properties of multicomponent fluids over a wide range of temperatures and pressures, including the critical region.

Candidates should possess a PhD in Chemical Thermodynamics and have experience in the experimental and theoretical study of transport properties and phase equilibria. Practical experience in computer programming and high pressure technology is essential.

An attractive remuneration package includes non-contributory pension scheme, subsidised restaurant, on-site sports and social facilities and relocation assistance where appropriate.

Please write, quoting reference SR.2432 to:
Mrs. Anne Hendry, The British Petroleum Company plc, BP Research Centre, Chertsey Road, Sunbury-on-Thames, Middlesex TW16 7LN. (2056)A

BP Research
SUNBURY-ON-THAMES

SANDOZ RESEARCH INSTITUTE
Vienna, Austria

is one of three research centres of the Swiss-based international Pharma-group SANDOZ. Our task is the development of new pharmaceuticals for the prophylaxis and therapy of infectious diseases and cancer.

We are seeking a

TUMOUR IMMUNOLOGIST
(PhD or MD)

The successful candidate will study the influence of biological response modifiers on tumour growth and metastasis both in vitro and in animal models.

A minimum of two years postdoctoral experience in the field of tumour research and animal experimentation is expected.

Research facilities and working conditions are excellent. Salary will be negotiable, commensurate with experience and qualifications. Other benefits include a non-contributory pension plan and full reimbursement of moving expenses.

Please send application with curriculum vitae, a list of publications and the names of referees to

SANDOZ

SANDOZ Research Institute
Personnel Department
Brunner Strasse 59
A-1236 Vienna/ Austria

(W989)A

NATIONAL MUSEUMS OF KENYA
DIRECTOR OF RESEARCH
INSTUTE OF PRIMATE RESEARCH (IPR)

The National Museums of Kenya invite applications from active research scientists with managerial experience for the post of Director of Research, IPR. The Institute has recently moved to a new facility, has a staff of 60 including 6 postdoctoral researchers and carries out studies in primate reproduction, tropical disease and conservation.

The Director of Research will be responsible for furthering the scientific reputation of the Institute, for attracting research grants (currently in excess of $300K per year), for maintaining research and training links with other agencies within Africa and internationally; and will wish to pursue his own research programme as well as managing the development of the Institute. Experience of successful research in developing countries would be an advantage.

The salary and conditions of service will reflect the importance of this post within the National Museums of Kenya. Applications, with detailed c.v. and the names of three referees, should be addressed to: R.E. Leakey, Chief Executive, National Museums of Kenya, PO Box 40658, Nairobi, Kenya, to arrive before 1 July 1984. (2016)A

Chisholm Institute of Technology
MELBOURNE, AUSTRALIA.

Dean
Faculty of Technology
(Ref. 84/38)

Applications are invited from suitably qualified persons for appointment as Dean, Faculty of Technology, Chisholm Institute of Technology. The appointee will be a member of the senior management team and will report to the Director.

Chisholm Institute of Technology is a multi-discipline post-secondary education institution offering a wide range of tertiary, specialist and continuing education courses on two campuses with an enrolment in advanced education courses in excess of 6,500. In January 1984 the Schools of Applied Science, Computing & Information Systems and Engineering were amalgamated to form a new Faculty of Technology which offers associate diploma, diploma, degree, post-graduate diploma and master's courses in a wide range of scientific and technological disciplines to approximately 2,100 students.

Applicants should preferably possess a higher degree, an outstanding record of achievement in academic and/or industrial research and development, together with a demonstrated ability to lead creatively the education and research activities of a multi-disciplinary Faculty.

It is expected that the appointee will be able to identify with the projected thrusts of the Faculty, provide academic leadership and foster and extend established links with professional associations, industry and government agencies.

The terms and conditions of appointment will be a matter for negotiation but it is envisaged that the successful applicant will be offered a five-year appointment at Head of School I, currently $A48,997 p.a. (to be supplemented by the recently approved National Wage Case 4.1% and the Academic Salaries Tribunal 5% salary increase). Other benefits are applicable.

A Position Specification is available from the Staff Branch, telephone (03) 573 2362.

Written applications, in duplicate, quoting the reference number, and including personal and career details, and the names and addresses of three referees, should reach the Staff Officer by 16 July, 1984. (W987)A

Caulfield Campus,
900 Dandenong Road, Caulfield East, 3145.
Victoria, Australia. Phone (03) 573 2222. 62674

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
(University of London)
Keppel Street, WC1E 7HT

DEPARTMENT OF MEDICAL HELMINTHOLOGY

A "NEW BLOOD" LECTURESHIP

is available for a postdoctoral scientist under the age of 35 years to work on the genetic engineering of helminth antigens. Applications are invited from candidates with experience in gene cloning and/or immunology to lead studies on the genetic engineering of helminth antigens for defined antigen vaccines and immunodiagnosis, with particular emphasis on schistosomiasis and filariasis. The work is to be carried out in collaboration with the School's Wolfson Unit of Molecular Medical Microbiology and Parasitology, and will be an integral part of the research of well-established groups at the School studying the immunology of these infections.

Salary on appointment in the range £8,530 — £14,125 (under review) plus £1,186 London Weighting per annum, with normal superannuation benefits.

Applications, consisting of full curriculum vitae and naming two professional referees, should be sent to the School's Senior Assistant Secretary by 14 June 1984. (2039)A

SCOTTISH CROP RESEARCH INSTITUTE
Invergowrie,
Dundee DD2 5DA

HIGHER SCIENTIFIC OFFICER

A Plant Physiologist is required in the Department of Physiology and C_7 Production to join a group of physiologists studying growth and development of the potato at both crop and single plant level.

The appointee will investigate the carbohydrate balance of the growing tuber, factors affecting tuber dry matter content, and the influence of growing conditions and genotype on subsequent sugar accumulation during storage.

Qualifications: A first or upper second class honours degree with at least two years post qualifying relevant research experience which may be a course of research leading to a PhD degree.

Salary: £7,149-£9,561.

Non-contributory superannuation.

"The Institute is an equal opportunities employer".

Applications giving full details of age, education and experience together with the names of two referees should be lodged by 15 June, with Mrs J A Shepherd, the Personnel Officer of the Institute, from whom further details may also be obtained. (2053)A

RECOMBINANT DNA RESEARCH
Group Leader

Connaught Laboratories Limited is seeking a distinguished scientist to lead an active and growing team in the field of molecular genetics, oriented towards research into products of biological and medical signficance. The successful candidate will have extensive research experience and an outstanding publication record, demonstrating scientific creativity and a broad background in all aspects of molecular biology. Previous experience as a Group Leader, Project Leader or Principal Investigator is essential, while industrial experience is desirable but not essential.

Connaught Laboratories has been a major producer of biologicals for human and veterinary use for seventy years. Our research facilities are located in Toronto, Ontario. The Company offers a competitive salary and comprehensive benefits package including a bonus scheme, along with the opportunity to work in a stimulating, multidisciplinary research environment.

Curriculum vitae and names of three references should be sent in confidence to Dr Keith J. Dorrington, Vice-President, Research and Technology, Connaught Laboratories Ltd., 1755 Steeles Avenue West, Willowdale, Ontario, Canada M2R 3T4. (NW521)A

AN EQUAL OPPORTUNITY EMPLOYER

CONNAUGHT
where service complements research

UNIVERSITY OF LONDON

King's College London
Queen Elizabeth College
Chelsea College

'NEW BLOOD' LECTURESHIP IN PHYSICS

Applications are invited for an appointment from 1 October 1984 under the 'new blood' scheme in the research field of 'Inverse source problems in physics'. The successful candidate will contribute to research, already strongly represented in the Image Analysis Group led by Professor R E Burge, on inverse problems in scattering by electromagnetic and matter waves, eg, rough surfaces scattering, solution to the phase problem, super-resolution. The three Colleges are in the process of merging, and the Physics Department is being located on the Strand site of King's College.

Candidates should be less than 35 years of age on 1 October 1984 and must not currently hold a permanent university appointment in the United Kingdom. Salary on appropriate point on scale £7,190—£14,125 pa, plus London allowance of £1,186 pa.

Further particulars and application forms from the Secretary, Queen Elizabeth College, Campden Hill Road, London, W8 7AH. Tel 01-937 5411 ext 246. Closing date 4 June 1984. (2035)A

UNIVERSITY OF DURHAM

DEPARTMENT OF PHYSICS

Applications are invited from physicists qualified to PhD level for appointment from 1 October 1984 or earlier by arrangement as

SENIOR RESEARCH ASSISTANT/SENIOR EXPERIMENTAL OFFICER

The successful applicant will join a team working on Very High Energy Gamma Ray Astronomy and will contribute to the construction, commissioning and deployment of a large telescope for the detection of 200GeV gamma rays using the ground-based atmospheric Cerenkov radiation technique.

The appointment would suit an experimental physicist/astronomer. Experience in electronics, computing or observational astronomy would be an advantage.

Salary will be on the 1A scale £7,190-£11,615 pa with superannuation and will involve working away from Durham for short periods.

Applications (3 copies) naming three referees should be sent by 15 June 1984 to the Registrar, Science Laboratories, South Road, Durham DH1 3LE from whom further particulars may be obtained. (2057)A

GRADUATE ASSISTANT
Biochemistry/ Microscopy

The Friedrich Miescher-Institute has a vacancy for a graduate assistant to work on the development of microscopic methods for immunohistochemistry using monoclonal antibodies and in situ hybridization using cDNA probes. A good background in biochemistry is essential. Although familiarity with histochemical procedures is also desirable, training in microscopic techniques will be given where necessary. The Institute possesses excellent facilities for electron microscopy and allied techniques and the appointment also involves responsibility for their maintenance.

The position offers the possibility of long-term employment in a research environment with a minimum of routine work.

Applications, including cv, should be addressed to Dr A. Matus, Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland. (W990)A

POSTDOCTORAL POSITION (Research Associate) available to study the binding of amphiphilic environmental chemicals to biological and model membranes and the relationship of binding to membrane activity. Requires PhD in biochemistry or other area of relevant training. Research background in membranes, binding studies, and liposome preparation highly preferred. Salary range $15,500-$17,000. Send résumé and three letters of recommendation before June 15, 1984 to: Dr Terry L. Miller, Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331. An Equal Opportunity/Affirmative Action Employer; complies with Section 504 of the Rehabilitation Act of 1973. (NW527)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

An SERC funded postdoctoral position to work on the mechanism of exocytosis in mast cells is available for one year from October 1984.

Salary will be in the range £8530-£10,710 according to age and experience.

Applications, including a curriculum vitae with list of publications should be made to Dr. J.C. Metcalfe at the Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW. (2046)A

THE UNIVERSITY OF ASTON IN BIRMINGHAM

FACULTY OF LIFE & HEALTH SCIENCES

Applications are invited for the following appointments which have been awarded by the University Grants Committee as part of its 'New Blood' initiative.

Department of Ophthalmic Optics

"NEW BLOOD" LECTURESHIP

(Ref: 605/6)

A vision scientist is required to join a multi-disciplinary team of researchers in the Clinical Neurophysiology Unit. The appointee will work on theoretical, physiological and pathological aspects of eye-movements and will also contribute to the Undergraduate Honours Degree Course in Ophthalmic Optics.

The Department is seeking a young person of exceptional promise. The ideal candidate will have a record of significant publications and the ability to work in a creative group of colleagues on research, teaching and the development of new techniques for clinical investigation.

Department of Pharmacy

"NEW BLOOD" LECTURESHIP IN ADVANCED DRUG-DELIVERY SYSTEM DESIGN (Ref: 606/6)

We seek to attract a young person of exceptional promise at present working in any of the basic or pharmaceutical sciences and motivated to make an original contribution within the area of advanced drug-delivery system design.

The successful candidate will be expected to collaborate with members of the Drug Development Research Group and to initiate new research projects on the design of drug-delivery systems appropriate for the novel molecules and therapeutic strategies engendered by recent advances in molecular biology and genetic technology.

The department proposes to co-ordinate its research activities within a Research Institute framework and concentrate its efforts in innovative and commercially relevant projects. The annual recurrent research budget of the Department from external sources is in excess of £600 K and considerable resources will be mobilised to support the new post.

The appointments will be for a period of three years initially, with the possibility of renewal or subsequent transfer to a continuing appointment. Initial salary will be within and up to the maximum of the range £7,190 to £14,125 per annum.

Application forms and further particulars may be obtained from: **The Staff Officer (quoting the appropriate reference number), University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET. Tel: 021-359 3611 Ext. 4564.**

Closing date for the receipt of applications is 15th June, 1984 (2062)A

UNIVERSITY OF ABERDEEN

DEPARTMENT OF BIO-MEDICAL PHYSICS & BIO-ENGINEERING

'NEW BLOOD' LECTURESHIP FOR MEDICAL IMAGING (NMR)

Applicants invited to join internationally known NMR Imaging Team of 11 total (8 MRC supported) led by Professor Mallard, Dr J M S Hutchison and Dr M Foster. The successful applicant will be particularly concerned with developing techniques for the detection and localisation of atheroma and the imaging of materials containing fluorine; also join in teaching of medical imaging at all levels, including MSc.

Salary will be on the Lecturer scale £7,190-£14,125 per annum (under review) with appropriate placing.

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 8 June 1984. (2013)A

UNIVERSITY OF GLASGOW

DEPARTMENT OF GENETICS

POSTDOCTORAL POST

A Postdoctoral position is available in the Department of Genetics for 3 years from October 1984, to work with Dr Howard Jacobs on the Isolation and Characterisation of Nuclear-coded Mitochondrial Genes from Mammalian Cells. This research is funded by an MRC Project Grant, and salary will be at the appropriate point on Range 1A of the scales for Research and Analogous Staff, plus USS benefits.

Applicants should be familiar with somatic cell genetics and/or recombinant DNA techniques.

Applications, including full curriculum vitae and the names of two referees, should be sent to Dr H T Jacobs, Department of Genetics, University of Glasgow, Glasgow G12 8QQ before 31st May 1984.

In reply please quote Ref No 5242M. (1903)A

M.R.C. CLINICAL RESEARCH CENTRE
(NORTHWICK PARK HOSPITAL)
WATFORD ROAD, HARROW,
MIDDLESEX HA1 3UJ

POST-DOCTORAL FELLOW in MOLECULAR BIOLOGY

Applications are invited from science graduates for a three year post-doctoral position in the molecular medicine research group to work on the molecular basis of atherosclerosis. A minimum of 3 years' experience in molecular biology is essential.

Salary scales are related to those for University non-clinical academic staff and salary on appointment will depend on age and experience. Informal enquiries may be made to Dr. James Scott (Head of Research Group) on 01-864 5311 ext 2257.

Application forms and further details available from Miss J Cottam, Personnel Section, ext 2673 quoting reference 111/ 1/ 3981.

Closing date for applications 7th June 1984. (2015)A

UNIVERSITY OF OXFORD

DEPARTMENT OF BIOCHEMISTRY
BIOMEDICAL NMR GROUP

GRADUATE RESEARCH ASSISTANT
(REF: BT 104)

Applications are invited from graduates in electronic engineering, physics or allied subjects for the above post. The research, funded for four years by a programme grant, is concerned with biochemical and clinical investigations using NMR spectroscopy. The multi-disciplinary group, under the direction of Dr G K Radda, FRS, is located in the Department of Biochemistry and in the medical Facility at the John Radcliffe Hospital. The successful candidate will be expected to participate in developing NMR spectroscopy and imaging techniques for human studies.

Salary: on the scale £6310-£8530.

Applications: Including names and addresses of two referees, not later than the 9th June 1984 to: The Administrator, The Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU.

(2026)A

UNIVERSITY OF WYOMING

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL POSITION

available for two (2) years to study either the biochemistry of human mixed cryoimmunoglobulins or the spatial relationships among crystallins in the mammalian lens. Experimental approaches include spectroscopy (fluorescence energy transfer, CD, photoacoustic FTIR), differential scanning calorimetry and chemical crosslinking. No previous experience in these areas is necessary but an interest in applying physical approaches to biomedical problems is essential.

Please send CV and have three (3) letters of reference forwarded to: Dr. Russ Middaugh, Department of Biochemistry, University of Wyoming, Box 3944, University Station, Laramie, WY 82071, USA. Telephone: (307) 766-2273.

(NW529)A

BIRBECK COLLEGE
(University of London)

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH OFFICER IN PHOTOCHEMISTRY AND ENERGY TECHNOLOGY

Photochemistry will be used to probe the mechanism of free radical reactions in coals, with particular emphasis on novel processes for coal liquefaction. This SERC Research Officer post is available for two years from September 1984. Appointment will be made on scale £8080-£8530 pa (under review) plus £1186 pa London Weighting. Applicants should have a background in either organic photochemistry or coal chemistry. Applications in duplicate to include full CV and names and addresses of two referees should be sent to the Assistant Secretary (Personnel) (N) Birkbeck College, Malet Street, London WC1E 7HX. Further information can be obtained from Dr J R Darwent, Department of Chemistry (01-580 6622 ext 223). (2037)A

UNIVERSITY OF OXFORD

NUFFIELD DEPT OF CLINICAL MEDICINE

John Radcliffe Hospital

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post to join an existing team working on the elucidation of the molecular basis of Duchenne and Becker muscular dystrophies. The project is supported by the Muscular Dystrophy Group of Great Britain. Previous experience of DNA recombinant technology is desirable but not essential.

Salary: Research Staff 1A £7,190 — £11,615. Position available immediately. Application form and job description from: Departmental Administrator, Nuffield Dept of Clinical Medicine, Room 5801, John Radcliffe Hospital, Headington, Oxford OX3 9DU.

Closing date for applications: 1st June 1984. (2030)A

TRUDEAU INSTITUTE

A Postdoctoral research position in infectious disease

is currently available to study interactions between several intracellular microbial pathogens and the cellular defenses in both normal and immunosuppressed animals. Current research is directed towards understanding cellular interactions responsible for the modulation of delayed-type hypersensitivity and acquired antimicrobial resistance during the development of chronic systemic disease in inbred mice. Candidates should have a strong background in infection-immunity or in immunology.

The Trudeau Institute is a private, not-for-profit basic medical research institution situated on the shores of Lower Saranac Lake, about a mile from the village of Saranac Lake and 10 miles from Lake Placid, NY. On-campus housing is available. Salary is at appropriate NIH levels.

Send curriculum vitae and names of three references to: Dr Frank M. Collins, Trudeau Institute, P.O. Box 59 Saranac Lake, N.Y. 12983. An Equal Opportunity Employer.

(NW525)A

MIPCORP

RESEARCH MANAGER

Cultivation of Algae. manage laboratory and field cultivation of spirulina algae; oversee research of new strains, conduct studies of growth rate and use of nutritionally important and other algae for mass culture/large scale algae ponds in Desert Hot Springs area. MS in Microbiology or Botany, and 4 years experience in culturing of algae required. $25,000/yr, 40/hrs/wk. Send résumé to MIPCORP, PO Box 867, Boulder Creek, CA 95006.

(NW528)A

UNIVERSITY COLLEGE LONDON

"New Blood" Lectureship

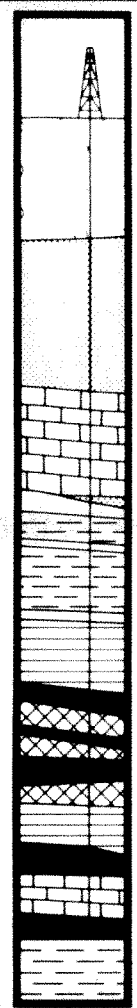
CELL BIOLOGY

Applications are invited for the above Lectureship in the Department of Experimental Pathology under the UGC's "New Blood" initiative. The post is available from 1 October 1984 and candidates should normally be aged 35 or under.

Applicants should be in the position of initiating and carrying out a research programme in the field of the transport and chemical processing of viral proteins within cells.

Salary in range £7,190-£14,125 per annum (under review) plus £1,186 per annum London Allowance.

Applications (no forms), including a full cv and names and addresses of two referees, should be sent to Assistant Secretary (Personnel), University College London, Gower Street, London, WC1E 6BT by no later than 8 June 1984. (2040)A



Stratigraphy Branch
BP Research Centre, Sunbury

Geologist – Computer Analyst

The Stratigraphy Branch of the Exploration and Production Division at the BP Research Centre in Sunbury requires a geologist with a strong and proven interest in computing. Applicants should have a first degree in geology and substantial experience of programming and using computers. Experience in the use and administration of data bases would be an advantage.

The successful applicant will work within the Computer Applications Section which currently supports two areas of work: palaeontology and stratigraphy. In the former area, the main computing project underway is the building of a palaeontology data base and associated applications programs for retrieval and graphical display of data. The principal stratigraphy computing project is the development of a linked data base — mapping system for the production of palaeoreconstructed maps with super-imposed geological information.

The present requirement is for a person with capabilities in two areas: data base administration and applications programming. The successful applicant will be responsible for maintaining the palaeontology data base and for maintaining links with other databases. A strong interest in the organisation and maintenance of large amounts of diverse data is therefore an essential prerequisite for the job. Additionally, the post holder will undertake applications programming tasks covering a range of disciplines within petroleum exploration geology.

The salary and benefits package reflects that of a major international business and includes a non-contributory pension scheme, subsidised restaurant and on-site sports and social facilities.

Please write, enclosing full curriculum vitae and quoting reference number STR 2617, to: Mrs. A. Hendry, BP Research Centre, Sunbury-on-Thames, Middx. TW16 7LN. (2054)A

BP Exploration Limited

PUBLIC HEALTH LABORATORY SERVICE BOARD

PHLS CENTRE FOR APPLIED MICROBIOLOGY AND RESEARCH

MICROBIAL TECHNOLOGY LABORATORY

BASIC GRADE MICROBIOLOGIST

required to join a group of scientists studying the microbial sequestration of heavy metals and the microbial degradation of chlorinated organic compounds. The successful applicant should preferably have a PhD in microbiology or biochemistry.

The post is funded by a research contract from the Department of the Environment and is tenable until July 1986.

Salary will be in the range of £6277 — £8349.

National Health Service terms and conditions will apply.

Applicants should send a detailed curriculum vitae and the names of two academic referees to Mrs M. Bushby, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wiltshire SP4 0JG.

Closing date: 1 June 1984. (2041)A

RESEARCH SCIENTIST, to study the anion transport system in normal, fetal and malignant liver cells. Must have three (3) years experience in membranes and Immunology. Must have PhD in Biochemistry. Salary $19,000/yr. Job site in L.A., California. Send this ad and your resume to job no.4077, P.O. Box 865, Sacramento, CA 95804 no later than June 8, 1984. (NW538)A

UNIVERSITY OF CAMBBRIDGE

DEPARTMENT OF PHYSICS

POLYMER SCIENCE RESEARCH

It is wished to fill a vacancy from 1 October for a postdoctoral scientist with qualifications in physics, chemistry or materials science to work on a SERC supported research project applying TEM techniques to a range of problems in polymer science. Previous experience of research using transmission electron microscopy is desirable.

Salary range £7190 to £9425 p.a.

Applications should be addressed to Mr J Deakin, Cavendish Laboratory, Madingley Road, Cambridge CB3 OHE, by 16 June, quoting ref. AD. (2008)A

UNIVERSITY OF EDINBURGH

POSTDOCTORAL POSITIONS IN MOLECULAR BIOLOGY

Scientists with expertise in immunochemistry/hybridoma technology and gene manipulation are required to join a team engaged in identification and cloning of genes for clinically important antigens of a human malaria parasite.

Send cv and the names of two referees to: Dr J G Scaife, Department of Molecular Biology, Darwin Building, Edinburgh University, King's Buildings, Mayfield Road, Edinburgh EH9 3JR. (1974)A

PHOSPHOINOSITIDE METABOLISM: Postdoctoral position. Starting summer-fall 1984. Innovative, independent, recent PhD in biochemistry with experience in lipid chemistry preferred. Our laboratory has been investigating the involvement of PI metabolism in calcium gating. Minimum salary $16,000. Send résumé to: Dr S E Rittenhouse, Hematology Research MRB 216, Harvard Medical School, 75 Francis Street, Boston, MA 02115.

(NW532)A

CSIRO

AUSTRALIA

PHYSICIST

$A24,344-$A35,806

DIVISION OF TEXTILE PHYSICS

RYDE NSW

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Textile Physics is a member of the Institute of Industrial Technology.

FIELD: Fabric filtration.

GENERAL: The Division of Textile Physics has a program concerned with research into various aspects of the use of textiles for the filtration of dust from gas streams, especially flue gases in, eg, smelters and power stations.

DUTIES: As a member of the Fabric Filtration Group, undertake research on the relevant properties of textiles, the interaction of dust with the textiles, and adhesion of dust particles within the collected dust cake.

QUALIFICATIONS: A PhD degree or equivalent qualifications with major interest in applied physics and mathematics. Knowledge and experience in fields such as adhesion studies involving textiles or polymers in general would be an advantage.

TENURE: Indefinite with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal and professional details, the names of at least two referees and quoting reference No A1167, should be directed to:

The Chief
CSIRO Division of Textile Physics
338 Blaxland Road
RYDE NSW 2112
AUSTRALIA

by June 15th, 1984.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W985)A

SOUTH AFRICA

Applications are invited from suitably qualified persons, regardless of sex, race, colour, or national origin for appointment to the following positions at the University of Witwatersrand, Johannesburg.

ASSOCIATE PROFESSOR/SENIOR LECTURER
Department of Genetics

Applicants should have a PhD in Genetics or an equivalent qualification in an appropriate related field such as Microbiology or Biochemistry together with a working knowledge of Genetics.

The successful candidate will be expected to teach topics in Prokaryotic Molecular Genetics and Genetics of Micro-organisms to third-year and Honours students and to undertake supervision of post-graduate students in these fields. A knowledge of recombinant DNA technology and an interest in the molecular genetics of higher plants will be a recommendation.

LECTURER
Department of Chemistry

Applicants should hold a PhD and have developed research interests at post-doctoral level in the general field of organic chemistry.

Salary level will be determined according to the qualifications and experience of the successful candidates and the ranges include a 12% pensionable allowance.*

Associate Professor:	R23,785 – R29,248 per annum.
Senior Lecturer:	R18,543 – R26,930 per annum.
Lecturer:	R14,178 – R24,834 per annum.

Benefits for elegible applicants include an annual bonus • pension • medical aid facilities • housing subsidy • remission of fees for dependants attending the University • travel assistance • removal costs • settling-in allowance.

A detailed information sheet can be obtained from Geof Henning, South African Universities Office, Chichester House, 278 High Holborn, London WC2V 7HE, quoting ref.1193 clearly on the envelope.

* £1 = R1.79 approx. (2044)A

UNIVERSITY OF THE WITWATERSRAND

POSTDOCTORAL POSITION IN LIQUID STRUCTURE RESEARCH

UNIVERSITY OF GUELPH

The liquid structure group in the Physics Department is seeking a Postdoctoral Research Fellow to work in the area of structure and dynazmics of gases and liquids. Current programs include measurements and simulation of the dynamics of dense noble gases and of Lorentz gas model systems, structural measurements on water and other molecular liquids, and the structure of water at the clay interface. As well as operating two neutron diffractometers at McMaster University and Chalk River Nuclear Laboratories, the group has an X-ray diffractometer and is pioneering the development of a liquids Gamma-ray diffractometer. Occasional trips to major facilities in the U.S. and Europe are also made. The applicant should have a rent PhD in experimental physics or chemistry or a related discipline and should be conversant in computer programming.

Please send applications, including resumé and names of at least two referees to: P A Egelstaff or A K Soper, Department of Physics, University of Guelph, Guelph, Ontario, Canada N1G 2W1. (NW524)A

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF PHYSIOLOGY

'NEW BLOOD' LECTURESHIP

Applications for the above post are invited from candidates with experience in cardiovascular physiology and an interest in pursuing research relating to the control of cortical blood flow. Candidates wishing to visit the department or make preliminary enquiries should contact Professor A M Sillito (0222 44211 ext 2002).

Candidates should be less than 35 years old, and must not currently hold a permanent University appointment in the UK. Appointment to commence on the 1st October, 1984, and will be made at the appropriate point on the lecturer scale £7,190-£14,125 per annum.

Applications (3 copies), with curriculum vitae, together with the names and addresses of two referees should be forwarded to the Vice-Principal (Administration) and Registrar, University College, PO Box 78, Cardiff CF1 1XL from whom further particulars my be obtained. Closing date 14th June, 1984. Ref: 2777. (2024)A

ST MARY'S HOSPITAL MEDICAL SCHOOL
(University of London)
Norfolk Place,
London W2 1PG

Applications are invited for the post of

LECTURER IN PHARMACOLOGY

The appointment calls for experience in the teaching of physiological and receptor pharmacology and the appointee would be expected to take immediate responsibility for the teaching of these areas within the pre-clinical course. An interest and experience in the application of computing techniques in both teaching and research is also desirable. The appointee will be expected to pursue a research programme in the general area of receptor pharmacology and also to develop BSc teaching options in this area.

Applications in duplicate in the form of a full cv with names and addresses of two referees should be sent to the Assistant Secretary (Personnel) at the above address by 1 June. (2032)A

UNIVERSITY COLLEGE LONDON

POSTDOCTORAL POSITION IN ELECTROPHYSIOLOGICAL ANALYSIS OF SYNAPTIC MECHANISMS

Applicants are invited for work on neurotransmitter-operated ion channels. At present this work involves mainly single ion channel recording from neuronal cells from peripheral autonomic ganglia, though other methods and other preparations may also be used. The laboratory has long term MRC support to David Colquhoun and Stuart G. Cull-Candy and is well equipped for this sort of work. Those who have applied for our recently advertised job in receptor reconstitution need not re-apply.

Please send applications to Professor D Colquhoun, Department of Pharmacology, University College London, Gower Street, London, WC1E 6BT. (2034)A

PLANT MOLECULAR GENETICS

A tenure-track position at Assistant/Associate Professor level is open immediately in the Genetics Department, NCSU. Position involves 70% research and 30% teaching. Individual expected to have strong training and expertise in molecular biology and to develop a high quality research program. Applications must be received by 15 July 1984. Send CV, statement of research and teaching interest and three letter of reference to: Dr John G Scandalios, Head, Department of Genetics, Box 7614, North Carolina State University, Raleigh, NC 27695-7614.

North Carolina State University is an affirmative action/equal opportunity employer. (NW534)A

MEDICAL RESEARCH COUNCIL SCIENTIST GRADE II Ref MS/5/84

Applications are invited for a short term non-clinical scientific post tenable for three years. Candidates of immediately post-doctoral status will be preferred. The appointee will work as a member of a group on an established project concerned with cytogenetic and molecular aspects of malignancy in cultured human B lymphoid cells. Experience in molecular biology will be an advantage but is not essential. Salary according to age and experience will be on MRC scales, and applications with the names of two referees should be forwarded to Mr A Douglas, Administrator, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU by 4th June 1984, quoting reference MS/5/84. (2019)A

OXFORD POLYTECHNIC

DEPARTMENT OF GEOLOGY AND PHYSICAL SCIENCES

TEMPORARY TECHNICIAL — GEOLOGY REF: S/18

Temporary technician required from August/September 1984 initially for one year with the probability of a further year. For the first year duties will include cataloguing and curating of geological specimens and preparation of laboratories for practical classes.

For the second year assistance would be required with the preparation of thin-sections for teaching, research and student projects. Appropriate qualifications in Geology (eg BTEC, HNC or Degree) and/or experience in the areas mentioned desirable.

Salary according to qualifications and experience in the range Scale 3/4:

Scale 3 £5640-£6135
Scale 4 £6264-£7005

Plus qualification allowance where appropriate.

Application forms and details available from the Staffing Department, ext 364. Closing date: 31.5.84.

Gipsy Lane, Headington, Oxford OX3 OBP. Telephone Oxford 64777. (2064)A

CENTRE FOR REPRODUCTIVE BIOLOGY, UNIVERSITY OF EDINBURGH

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

LECTURER IN REPRODUCTIVE BIOLOGY

Applications are invited from scientists under 35 years of age for the above post created under the New Blood Scheme. Persons already holding a permanent university appointment in the UK are not eligible. The successful applicant will be expected to undertake research related to the biology of gametes in collaboration with a team of scientists and clinicians involved with human in vitro fertilization. Teaching responsibilities include the supervision and training of PhD students and research fellows. There are excellent opportunities for collaborative research with other scientists in the centre.

The salary will be at an appropriate point on the scale £10,710-£14,125 per annum.

Further particulars can be obtained from Professor David T Baird, Department of Obstetrics and Gynaecology, University of Edinburgh, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh, EH3 9EW.

Applications (12 copies) including curriculum vitae and giving the names and addresses of two referees, should be sent to The Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, EH8 9YL. By 30th June, 1984.

Please quote reference NB6 (84). (2049)A

TEMPLE UNIVERSITY OF MEDICINE DEPT. OF MICROBIOLOGY and IMMUNOLOGY — Applications are invited for two full time faculty positions (one at tenure track) at the Assistant or Associate professor level. We are searching for highly qualified individuals able to establish a productive independent research program. Candidates must have a Doctoral degree, extensive post-doctoral experience and publications in microbial genetics, molecular biology of host pathogen interactions or immunology. Successful candidates will also be members of the graduate faculty and will have responsibility for teaching both graduate and medical students. Currently the Department consists of 13 full time resident faculty members plus several joint and affiliated appointees, and operates active doctoral and post-doctoral programs. Numerous opportunities for interdisciplinary interactions and collaboration are available with individuals with mutual interest in the Department, the Institution, and the Philadelphia area. Send curriculum vitae and names of three references to Gerald D. Shockman, PhD, Chairman, Dept. of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA 19140. Equal opportunity, affirmative action employer. (NW537)A

BIOTECHNOLOGY

Celltech is one of the World's leading specialist biotechnology companies. Based in the UK. The Company is backed by four major City of London financial institutions. A strong scientific team has now been built up in the areas of recombinant-DNA and cell-hybridisation and continued expansion is envisaged; close links are also maintained with the academic community. The Company aims to exploit the potential of discoveries arising in the field of biotechnology, and commercial successes have already been adhieved in several areas.

CHIEF TECHNICIAN — Immunology

Further expansion of Celltech's activities in areas of immunology including production of monoclonal antibodies as well as cell produced factors that regulate the immune response is planned. This gives rise to a position for a highly skilled Tissue Culture Technician with very wide experience in many tissue culture applications such as both primary cultures and cell lines.

This person will have responsibility for operation of a new tissue culture laboratory, supervision and training of personnel as well as collaborate with scientists on a wide variety of scientific problems using the facilities. The successful candidate should have at least fifteen years' experience of tissue culture including growing of a large number of different kinds of rodent and human cells, growth and assay of a wide variety of viruses, cell fusion technology, diagnostic assays, protein separation techniques and assays. A part of the research programme involves growing and cloning of different cells. The training of staff in all these areas is part of the responsibility of this position.

Since it is expected that Celltech will expand its laboratories, experience in design and setting up of similar tissue culture laboratories is essential.

Please quote reference number 202.

GROUP LEADER

We require a group Leader to run a section of several Tissue Culture Technicians who produce and assay monoclonal antibodies. Applicants should have at least 5 years' post-doctoral experience with a good grounding in immunology.

The successful applicant will be required to interact with other research groups inside and outside the company and with commercial divisions of the company and will also be expected to carry out bench work in addition to the above managerial duties.

Please quote reference number 203.

To apply, please send a full C.V. quoting the appropriate reference number to:-

Jane Walley, Personnel Officer, Celltech Limited, 244-250 Bath Road, Slough, Berkshire SL1 4DY.

(2065)A

CELLTECH

CSIRO
AUSTRALIA
GLASSY METALS RESEARCH RESEARCH FELLOW
$A24,344-$A30,038
DIVISION OF APPLIED PHYSICS LINDFIELD NSW

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Applied Physics is a member of the Institute of Physical Sciences.

The Division, with a staff of nearly 400, undertakes research in applied physics related to problems in industry and community, and collaborates with industry in exploiting promising developments. Research includes programs in optics; electric, magnetic and thermal properties of materials; discharges in gases; and the measurement of temperature. The Division is also responsible for the maintenance of the Australian standards of physical measurement.

The Division wishes to appoint a Research Fellow under the Australian National Research Fellowship Scheme to work on the production, properties and uses of glassy metals.

Under the National Research Fellowship Scheme, preference is given to Australian citizens or residents.

DUTIES: The appointee will join an existing research program and will take part in research aimed at producing new glassy metals and investigating their properties.

QUALIFICATIONS: A PhD degree in physics, metallurgy, materials science or engineering, or equivalent qualifications, plus proven research ability.

TENURE: A term of 3 years. Australian Government superannuation benefits are available.

APPLICATIONS: Stating full personal and professional details, the names of at least two referees, and quoting reference No M8668, should be directed to:

The Chief
CSIRO Division of Applied Physics
PO Box 218
LINDFIELD NSW 2070
AUSTRALIA

by June 15, 1984.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W988)A

UNIVERSITY OF STIRLING

DEPARTMENT OF BIOLOGICAL SCIENCE

POSTDOCTORAL RESEARCH FELLOW

Applications are invited from candidates who have recently graduated or expect to graduate in the near future with a PhD Degree in Biochemical or Chemical Sciences. The post, for two years, is funded by a grant provided by the multiple sclerosis society to Dr. P.J. Brophy and Dr. A. Watts of Oxford University. Candidates should have some experience in one or more of the following areas: Membrane Biochemistry, Organic Synthesis or NMR Spectroscopy. The research will be on Lipid-Protein interactions in Myelin. Salary within IA Scale which starts £7,190 per annum. It is expected that the successful applicant will be based in Oxford University.

Applications, together with the names of two referees, should be sent to the University Secretary, University of Stirling, Stirling FK9 4LA by Friday 8 June 1984. (2045)A

POLYTECHNIC OF CENTRAL LONDON

Faculty of Engineering and Science
SCHOOL OF BIOTECHNOLOGY

TWO LECTURESHIPS IN MICROBIOLOGY AND BIOCHEMICAL/CHEMICAL INSTRUMENTATION (LII or SL)

Applications are invited from well qualified and experienced scientists for the above posts tenable from 1st September 1984 or as soon after as possible.

Salary scales (under review): Lecturer II £8,202-£12,555; Senior Lecturer £11,670-£13,539(BAR)-£14,430 per annum inclusive of London Allowance.

Further particulars and application forms from the Personnel Office, Polytechnic of Central London, 309 Regent Street, London W1R 8AL. Closing date: 1st June 1984. (2043)A

ROCHE

The **Clinical Pharmacology** group of the **DEPARTMENT OF CLINICAL RESEARCH** is responsible for investigation of the **pharmacodynamics** and **pharmacokinetics** of new drugs in humans.

To expand our small team, we are looking for a

PHYSICIAN

with training in clinical pharmacology (internal medicine, pharmacology), and an interest in working in research.

This interesting field of work includes the planning, conducting, and evaluation of clinical pharmacology studies of research compounds in phases I-IV.

Pre-requisites are a knowledge of the methods of assessing drug effects in man, and/or pharmacokinetics, as well as a good command of German and English.

Interested applicants are requested to send complete application documentation to our Personnel Department, quoting reference Nature 50/83.

F. Hoffmann-La Roche & Co. Limited
Company, CH — 4002 Basle (W982)A

ROCHE

THE CHINESE UNIVERSITY OF HONG KONG

invites applications for the post of

LECTURER IN BIOLOGY

The appointee, who should hold a doctoral degree, is expected to undertake research and teaching on aspects of Physiology/Morphogenesis for Higher Plants. He will be a member of the Board of Studies in Biology and will participate in the general teaching (including a general course on Plant Biology) and all other activities of that Board.

ANNUAL SALARY: HK$135,780 — 154,140 by 2 increments BAR HK$163,260 — 227,100 by 7 increments. Exchange rate approximately: US$1 = HK$7.8, £1 = HK$12. Starting salary and grade will depend on qualifications and experience.

CONDITIONS OF SERVICE: Benefits include sick leave, vacation leave, long leave with pay, superannuation (University 15%, appointee 5%), medical benefits, education allowance for children and housing allowance for those whose annual salary is HK$144,960 or above, and for appointees on overseas terms, passage benefits for themselves and their dependents as well.

APPLICATION PROCEDURE: Applications should be made out in duplicate, giving full particulars, experience and the names and addresses of 3 persons to whom reference may be made, and sent together with copies of certificates/diplomas/testimonials and recent publications to the Personnel Section, The Chinese University of Hong Kong, Shatin, N.T. Hong Kong not later than *July 31, 1984*. Please quote reference number 14/509/2/84 and mark 'Recruitment' on cover. (W992)A

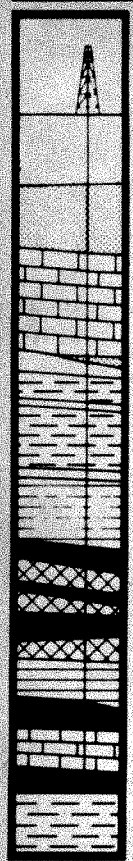
KINGDOM OF SAUDI ARABIA
KING SAUD UNIVERSITY
COLLEGE OF AGRICULTURE
RIYADH, SAUDI ARABIA

Qualified personnel are invited to apply for the following posts of FACULTY MEMBERS (Professors, Associate Professors, Assistant Professors), LECTURERS, DEMONSTRATORS, RESEARCH ASSISTANTS and TECHNICIANS for the academic year 1984 -1985.

Department	Specialization	Qualification
I. Staff members:		
(1) Food Science	a. Human nutrition metabolism. b. Dairy technology. c. Food analysis.	Ph.D., Teaching experience, Arabic speaking.
(2) Animal Science	a. Poultry nutrition.	-do-
(3) Plant Production	a. Cytogenetics.	-do-
(4) Ag. Economics & Rural Sociology	a. Farm management. b. Marketing of agricultural products.	Ph.D., Teaching experience, Associate or Professors only, Arabic speaking.
(5) Home Economics & Nutrition (Female only)	a. Textiles and costumes.	Ph.D., Teaching experience, Arabic speaking.
II. Lecturers:		
(1) Home Economics and Nutrition (Female only)	a. Plant production (ornamentals) b. Plant production (field crops).	M.Sc., Teaching experience, Arabic speaking.
(2) Plant Production	a. Plant production (vegetable crops).	-do-
(3) Animal Production	a. Animal production	-do-
III. Demonstrators:		
(1) Food Science	a. Fish and meat technology. b. Food microbiology. c. Food analysis.	B.Sc. "B" average or higher, past experience in teaching, research is preferred, Arabic speaking.
(2) Plant Production	a. Horticulture (vegetable crops).	-do-
(3) Agricultural Engineering	a. Agric. Engineering.	do-
IV. Research Assistants:	a. Food microbiology. b. Food chemistry and analysis. c. Horticulture (Pomology). d. Field Crops (Breeding).	Preferably M.Sc. with research experience in the field of specialization.
V. Technicians:	a. Human nutrition. b. Cereal technology. c. Analytical chemistry in the field of food science.	Experience in field of work, conducting and operating laboratory equipment. Experience and training in operating food and nutrition analyses equipment preferably IR, AAA, AA, GLC, HPLC. Good knowledge of English language.

Please apply to:
Dean, College of Agriculture,
King Saud University,
P.O. Box 2460,
Riyadh 11451,
Kingdom of Saudi Arabia.

(W986)A



Stratigraphy Branch
BP Research Centre, Sunbury

Geologist/Geophysicist

The Stratigraphy Branch of the Exploration and Production Division at the BP Research Centre in Sunbury requires a geologist/geophysicist with strong and proven interests in structural geology, plate tectonics, reconstructing past continental positions, palaeomagnetism and computing. Applicants should have a PhD degree in Geology or Geophysics, preferably with post doctoral research or industrial experience.

The successful applicant will work with the Stratigraphic Projects Section, which is responsible for developing and maintaining BP's expertise in plate reconstruction and structural geology to evaluate motions of continents through time, to help understand placement and timing of structures, basin development, and sediment accumulation in relation to plate motions, and can assist in developing and maintaining a computerised and cartographic system for palaeoreconstruction and palaeogeographic mapping. The person appointed will work as a member of a multidisciplinary team, and will be expected to interact closely with members of other BP groups directly involved in different aspects of petroleum geology.

The salary and benefits package reflects that of a major international business and includes a non-contributory pension scheme, subsidised restaurant and on-site sports and social facilities.

Please write, enclosing a full curriculum vitae and quoting reference number STR 2616, to: Mrs. Anne Hendry, BP Research Centre, Sunbury-on-Thames, Middlesex TW16 7LN.

(2055)A

BP **BP Exploration Limited**

UNIVERSITY OF TEXAS
HEALTH SCIENCE CENTER
AT DALLAS

DEPARTMENT OF RADIOLOGY

POSTDOCTORAL RESEARCH
NMR APPLIED TO TISSUE METABOLISM

Two positions are available to pursue research in physiology and metabolism using the non-invasive method of high resolution nuclear magnetic resonance. The laboratory is currently investigating several aspects of cardiac and brain metabolism using NMR techniques. Research projects in both basic studies and in clinical applications are available. The facilities of the NMR Laboratory are extensive and state-of-the-art and include a one meter bore system under development for clinical use. The laboratory also has an excellent staff to support various aspects of the research. Candidates should have training in biochemistry or physiology. Additional experience in physical and analytical chemical methods is desirable but not essential.

Please send a résumé and three letters of reference to: Ray L. Nunnally, PhD, Director of Biomedical Magnetic Resonance, Department of Radiology, University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235.

Equal Opportunity University.

(NW530)A

The European Molecular Biology Laboratory, a research Institute situated in Heidelberg, West Germany, invites applicants for the following vacancy at the Laboratory's Outstation at the Deutsches Elektronensynchrotron (DESY) site in Hamburg.

BIOPHYSICIST/PHYSICAL BIOCHEMIST

The successful candidate will interest collaboratively with visiting groups using time resolved X-ray diffraction and scattering methods for the study of poorly ordered systems (fibres, solutions, gels). In addition, he/she will be responsible for the instruments for these experiments and be given an opportunity to carry out a limited in-house research project.

Applicants must have a PhD and experience with X-ray structural methods.
Experience in computing will be an asset.
Fluent knowledge of English and of one or more of the other official languages of the EMBL (French, German) is a prerequisite.

An above average salary will be offered to the successful candidate.
Certain allowances are payable in addition, depending on personal circumstances.
An initial contract of 3 years' duration, which can be renewed, will be offered.

Please write briefly for an application form quoting reference No 84/22 to: EMBL, Personnel Section, Postfach 10.2209, D-6900 Heidelberg

EMBL

(W991)A

Department of Biology
Cell Biologist

The University of Calgary invites applications for a 3-year limited term position at the Assistant Professor level in the Department of Biology. The successful candidate will be expected to develop an active research program in the area of cellular/molecular biology of microbial eukaryotes, to teach an undergraduate course in Cell Biology and to participate in Biotechnology and Molecular genetics laboratory teaching. Applicants must have post-doctoral experience and strong credentials in molecular biology. Preference will be given to those with experience in recombinant DNA techniques.

The appointment will be open as of January 1, 1985, with an expected starting salary in the range of $29,700 to $35,000 per annum, based on the 1983-84 scale.

In accordance with Canadian Immigration requirements, this advertisement is directed to Canadian citizens and permanent residents of Canada.

Applicants are requested to submit a complete curriculum vitae, and to have three letters of academic reference forwarded, by September 1, 1984 to:

THE UNIVERSITY OF CALGARY

Dr. R.W. Davies
Department of Biology
The University of Calgary
2500 University Drive N.W.
Calgary, Alberta T2N 1N4

(NW520)A

UNIVERSITY OF THE WEST INDIES

Mona, Jamaica

pplications are invited for the ollowing posts:

PROFESSOR OF PURE MATHEMATICS, MONA, JAMAICA

Applicants of suitable stature in any area will be considered. Duties to be assumed as soon as possible.

LECTURER/ASSISTANT LECTURER IN BIOCHEMISTRY, MONA

It is desirable for teaching purposes that the successful applicant should have broad training in Biochemistry. Certain areas of research interest are particularly welcome, eg Plant Biochemistry, Toxicology, Nutrition, Immunology, Biotechnology. Duties to be assumed as soon as possible.

ELECTRON MICROSCOPIST

DEPARTMENT OF BOTANY, MONA

Successful applicant will be required to take charge of the Electron Microscope Unit comprising transmission and scanning electron microscopes, to supervise the work in the Unit and help users of the microscopes, and to train a limited number of technicians in methods of electron microscopy. He/she may also be required to teach a short course in his/her speciality. Preference may be given to applicants with interest in Biological Sciences. Duties to be assumed as soon as possible.

Salary Scales: Professor in range $38,340 — 47,194. Lecturer (Non-Medical) & Electron Microscopist $23,688 × 768 — 28,296 (B) × 825 — 33,246. Assistant Lecturer (Non-Medical) J$20,379 × 717 — 21,813 £1 = J$5.35 at 1/5/84).

Up to five full passages (economy class) plus baggage allowance up to J$900 on appointment and normal termination. Special allowance up to J$300 for shipment of academic books and teaching/research equipment on appointment. Unfurnished accommodation at 10% of pensionable salary or optional housing allowance of 20% of pensionable salary. Pension provision. Subsidised Health Service. Annual Book Grant. Annual Study & Travel Grant for self, spouse and three children. Transportation allowance to persons who operate a motor vehicle.

Detailed applications (three copies) giving full particulars of qualifications and experience, date of birth, marital status, and the names and addresses of three referees should be sent as soon as possible to the Registrar, UWI, Mona, Kingston 7, Jamaica. Applicants in the UK should also send one copy to the Assistant Secretary General (Appts), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF. Further details obtainable from either source. (W993)A

NATIONAL INSTUTUTE OF AGRICULTURAL ENGINEERING

PHYSICIST/ RESEARCH ENGINEER

The NIAE is respected internationally as a major centre of agricultural engineering research and development. It is engaged in a wide range of research projects, and one of its teams is working to increase the efficiency of greenhouse crop production.

A graduate Physicist/Research Engineer is now required to join this team engaged initially in developing mathematical models to be used to optimise the design of future greenhouses of novel shape and carry out research into new cladding materials and heating systems.

The appointment calls for a candidate with a first or upper second class degree in Physics/Engineering who can combine sound engineering and scientific principles with creative and innovative design.

Salary will be in the range £6,018-£9,561 per annum depending on experience and there is a non-contributory pension scheme.

The NIAE is situated in the beautiful countryside surroundings of Wrest Park, Silsoe, midway between Bedford and Luton. There are excellent working conditions and facilities both in Wrest Park mansion itself and in the advanced, purpose-built research laboratories and workshops.

Further details and an application form can be obtained from the Personnel Officer, National Institute of Agricultural Engineering, Wrest Park, Silsoe, Bedford MK45 8HS. Telephone: Silsoe (0525) 60000. Please quote ref 20/5. (2014)A

UNIVERSITY OF BRISTOL

DEPARTMENT OF PHARMACOLOGY

WELLCOME LECTURESHIP IN VETERINARY PHARMACOLOGY

Applications are invited from veterinary graduates preferably of not more than 35 years of age, for the post of Wellcome Lecturer in Veterinary Pharmacology tenable for a period of up to five years in the Department of Pharmacology. Persons holding permanent university appointments in the U.K. are ineligible for consideration.

The successful applicant will contribute to the teaching of pharmacology to veterinary and science students and will be expected actively to pursue a research project.

Salary will be in the range £7,190-£14,125 per annum depending on age and experience.

Applications, together with the names of two referees and a summary (up to 500 words) of research to be pursued, should be received not later than 16th June 1984 by the Registrar and Secretary, University of Bristol, Senate House, Bristol BS8 1TH, from whom further details may be obtained.

Please quote reference JF. (2022)A

CSIRO
AUSTRALIA
CHIEF OF THE DIVISION
DIVISION OF WILDLIFE AND RANGELANDS RESEARCH
INSTITUTE OF BIOLOGICAL RESOURCES
CANBERRA ACT

Applications are invited for the position of Chief, Division of Wildlife and Rangelands Research, from scientists who have an outstanding record of personal research achievement and leadership in an appropriate field of biological science.

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists.

The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The Division of Wildlife and Rangelands Research is one of the Divisions of the Institute of Biological Resources. Also in the Institute are the Divisions of Entomology, Forest Research, Horticultural Research, the Centre for Irrigation Research, and the Divisions of Plant Industry, Soils, Tropical Crops and Pastures, and Water and Land Resources.

The Division of Wildlife and Rangelands Research has its headquarters in Canberra, ACT, and laboratories at Darwin and Alice Springs, Northern Territory; Deniliquin, New South Wales and Helena Valley, Western Australia. The total Divisional staff of 204 includes 52 research scientists and 26 other professional staff.

The Division studies the ecology of animal and plant populations and processes controlling their stability, diversity, productivity and value to Australia in relation to their management and conservation. The Division's interests cover a wide range of Australia's ecosystems. Amalgamation of the Wildlife and Rangelands groups in 1982 gives the Division a special interest in rangelands research.

A Chief of a Division is responsible for the leadership, development, scientific direction and integration of the Division's research programs, and is encouraged to promote active collaboration with other Divisions, research bodies and agencies working in associated areas.

The salary for the position of Chief is negotiable, but will not be less than $A55,050 per annum. In addition, the position attracts an expenses of office allowance $A1,250 per annum.

Appointment to the Organization is for an indefinite period and Australian Government superannuation benefits are available. The postion as Chief is offered for a negotiable term typically of five to seven years, with subsequent options for a further term, if mutually desired.

Further information on the Division and its research is contained in a booklet available on request from the Secretary (Personnel) or the Secretary, Institute of Biological Resources at the address below. Dr M G Pitman, OBE, ScD, FAA, Director of the Institute of Biological Resources would be pleased to discuss the position with potential applicants. He would also be pleased to receive advice concerning this appointment from people with a particular interest in it. He may be contacted at the address below.

Applications stating full personal and professional details, the names of at least three professional referees and quoting reference No A5692, should be directed to:

The Secretary (Personnel)
CSIRO Australia
PO Box 225
DICKSON ACT 2602
AUSTRALIA

by June 15, 1984.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER *(W983)A*

UNIVERSITY OF DURHAM

DEPARTMENT OF GEOLOGICAL SCIENCES

Applications are invited for a New Blood Lectureship in Geophysics from 1 October 1984 or as soon as possible thereafter, to develop research programmes in the application of space geodesy to the study of earth structure and dynamics. Applicants must have previous experience in either geophysics or geodesy, and be willing to develop a broad interdisciplinary approach in both subjects.

Salary on the scale £7,190-£14,215 with supperannuation.

Applications (3 copies) naming three referees should be sent by 2 June 1984 to the Registrar, Science Laborabories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. (2010)A

Sedimentary Geochemists

We currently have two opportunities for enthusiastic and flexible scientists, who should be numerate, literate and enjoy putting together different types of results to attack exciting problems. They will join a small team investigating diagenetic processes and applying this knowledge to:

★ petroleum exploration and production
★ metalliferous minerals exploration

Candidates should have either:

- a PhD
  or
- an honours degree plus the ability and qualifications for post-graduate research but preference for a career in industry

  plus

  experience of (or be happy to learn fast) the techniques of:
  - stable isotope mass spectrometry
  - geochemical analysis
  - petrography/petrology

An attractive remuneration package includes non-contributory pension scheme, subsidised restaurant, on-site sports and social facilities and relocation assistance, where appropriate.

Please write, quoting reference SR 2816, to: Mrs. Anne Hendry, The British Petroleum Company plc, BP Research Centre, Chertsey Road, Sunbury-on-Thames, Middlesex TW16 7LN. (2070)A

BP Research SUNBURY-ON-THAMES

POSTGRADUATE RESEARCH IN BIOLOGY

(three posts)
Salary: £5,649 — £6,363 — Pay award pending

Applications are invited from candidates with, or expecting to obtain a good honours degree in Biological Sciences, for work in one of the following areas of research:-

1. Developmental changes in the distribution and diet of *Nucella lapillus* (L.)

2. Genetics of disease susceptibility in fish.

3. Lead uptake and translocation by different crop varieties.

4. Low temperature injuries in *Chlamydomonas reinhardii* (C.W. 15+)

Research Assistants are expected to register for a CNAA higher degree. Appointments are for a period of two years with a possibility of a third year (fixed term contract).

Further details and application forms to be returned by Friday 15th June 1984, can be obtained from the Personnel Office, Plymouth Polytechnic, Drake Circus, Plymouth PL4 8AA. Tel: (0752) 264639 (NB Please specify clearly for which project you are applying). (2028)A

Plymouth Polytechnic

UNIVERSITY OF BRISTOL

DIRECTOR, LONG ASHTON RESEARCH STATION

The University of Bristol invites applications for the post of Director of the Long Ashton Research Station which is the Department of Agriculture and Horticulture of the University of Bristol. The Station receives grant-in-aid from the Agricultural and Food Research Council. On appointment the Director will also assume responsibility for the Council's Weed Research Organisation near Oxford whose programme of work is being consolidated with that of the Long Ashton Research Station at Long Ashton.

The Director will be a Professor of the University of Bristol and Head of the Department of Agriculture and Horticulture.

The appointment, which is at Grade 4 of the new Open Structure of the Civil Service, carries a salary of £25,455 p.a. There is a non-contributory pension scheme.

Further particulars can be obtained from the Registrar and Secretary, Senate House, University of Bristol, Bristol BS8 1TH, to whom applications, together with the name and address of three referees, should be received not later than 14 June 1984.

The successful applicant may qualify for financial assistance with removal expenses. (2009)A

POSTDOCTORAL

position available to study the molecular mechanism of peptide action. Experience in hormone action, calcium as a second messenger, radioligand assays desirable. Salary: NIH scale. Send vita, statement of prior activities and interest, and names of three references to: Dr P Michael Conn, Professor and Chairman, Department of Pharmacology, College of Medicine, 2-512 BSB, University of Iowa, Iowa City, IA 52242. The University of Iowa is an Affirmative Action/Equal Opportunity employer. (NW523)A

MRC CELL BIOPHYSICS UNIT

Assistance is required for research on various aspects of neuronal cell biology under Dr J P Brockes. Interest in biochemistry and tissue culture is desirable, but not essential.

Qualifications: HNC, degree or equivalent.

Salary: According to experience in the range of £5491-£7747 plus £1042 London Weighting.

Apply in writing, giving the names and addresses of two professional referees to: Mr D M Drummie, MRC Cell Biophysics Unit, King's College, 26-29 Drury Lane, London WC2B 5RL.

Closing date: 4 June 1984. (2060)A

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY AND NUTRITION

DEMONSTRATOR

Applications are invited for the post of Demonstrator in the Department of Agricultural Biochemistry and Nutrition which will be tenable from 1st October 1984 for a period of 3 years. The person appointed will have the two-fold responsibility of running one or more laboratory courses in agricultural biochemistry and one in microbiology for students of Agricultural Science in the Faculty of Agriculture. The person appointed will also be asked to give a number of lectures on aspects of microbial biochemistry and will be expected to have a serious commitment to research.

Salary will be at an appropriate point on the Grade IB (Bar) scale: £6,310 — £8,530 per annum, according to age, qualifications and experience.

Further particulars may be obtained from the Senior Assistant Registrar (Establishments) (FP), The University, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU, with whom applications (3 copies) together with the names and addresses of three referees, should be lodged not later than 8th June 1984. Please quote ref N (2017)A

MONASH UNIVERSITY

Melbourne, Australia

DEPARTMENT OF BIOCHEMISTRY

FIXED-TERM LECTURER (3-5 YEARS)

Postdoctoral graduate for teachin medical or science students an participation in research in areas o (1) cloning and gene expression i yeast; (2) human interferon biology as well as genes and expression; (3 yeast mitochondril ATP synthetas complex; (4) monoclonal antibodie and protein chemistry. Enquiries t Professor A W Linnane.

Salary: $A24,353 — $A31,994 pa Applications including Ref n 30212A, curriculum vitae and 3 ref erees to the Registrar, Monas University, Clayton, Vic 3168 Australia, with a copy to th Secretary General, Association o Commonwealth Universitie (Appts), 36 Gordon Square, Londo WC1H 0PF, by 9/7/84, from whor Conditions of Appointment ar available.

An equal opportunity employer. (2068)A

Re-advertisement
UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF GEOLOGICAL SCIENCES

THREE RESEARCH POSTS IN ANTARCTIC MARINE GEOLOGY AND GEOPHYSICS

Applications are invited for (A) One Research Fellow, 1A post, to investigate abyssal sedimentation in the Weddell Sea, and (B) Two Research Associates, 1B posts, for work on subduction-related processes and the early stages of Gondwanaland breakup in the Scotia and Weddell Sea regions. Post (A) is for a Sedimentologist; appointees to posts (B) will have had training in geophysics.

Posts tenable for one year in the first instance, but the project is supported by a BAS Research Contract extending to 30/9/88. Salaries according to age and experience will be:—

On the Research Fellow 1A Scale £7,190-£11,615 plus superannuation.

On the Research Associate 1B scale £6,310-£8,530 plus superannuation.

For further particulars telephone 021-472 1301 ext 2559, quoting reference G2 (A) or G2 (B).

No formal application form. Three copies of application, including curriculum vitae and naming three referees, to Assistant Registrar, (Science and Engineering), P.O. Box 363, Birmingham B15 2TT, by 30th May 1984. (2048)A

UNIVERSITY OF GLASGOW

DEPARTMENT OF DERMATOLOGY

POST DOCTORAL RESEARCH ASSISTANT

Applications are invited for the above position in a small group studying the differentiation of normal and abnormal epidermis. The successful candidate will investigate factors controlling the proliferation and differentiation of the epidermis in vivo and in vitro with particular reference to the expression of cytoskeletal proteins.

The position is supported until 31st December 1988 by a programme grant from the Medical Research Council. Initial appointment will be at a salary of £7190 pa on range 1A of the salary scales for Research and Analogous Staff with superannuation benefit.

Applications enclosing a curriculum vitae and the names and addresses of two referees should be submitted to Dr D Skerrow, University of Glasgow, Department of Dermatology, Anderson College Building, 56 Dumbarton Road, Glasgow, G11 6NU. (2063)A

Please mention
nature
when replying to these advertisements

UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST THOMAS'S HOSPITALS
PAEDIATRIC RESEARCH UNIT

A Molecular Biologist is required to join a research group working on human genetics. The group is part of a multi-disciplinary academic research unit, with excellent laboratories, a scientific information service and full support facilities. The Unit includes the South East Thames Regional Genetics Centre. The molecular biology group has well established collaborative arrangements with a number of leading research centres. The applicant should have experience of recombinant DNA work, eg gene cloning, expression or structural analysis.

The appointment will be for an initial period of three years, with the possibility of an extension for a further period thereafter. Further particulars from Professor M Bobrow (01-407 7600 ext 2330).

Salary in range £7,190-£14,125 plus £1,186 London allowance plus Superannuation according to qualifications and experience.

Applications with a curriculum vitae and the names of two referees should be sent to the Secretary, United Medical and Dental Schools, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting ref PRU.

(2061)A

MEDICAL RESEARCH COUNCIL

LABORATORY OF MOLECULAR BIOLOGY
Cambridge

Applications are invited for a
CAREER NON-CLINICAL SCIENTIFIC

post with the Medical Research Council to conduct innovative research in biochemical crystallography particularly in the study of DNA protein complexes. The post is tenable in the Laboratory's Division of Structural Studies in the first instance.

Candidates should have experience in the area of the physical biochemistry of proteins and nucleic acids as well as in X-ray crystallographic structure analysis of large molecules.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff. Further information may be obtained from the Assistant Director (Administration) quoting reference SS/11.

Applications — including a full cv, publications list, the names of two professional referees and a statement of not more than 500 words describing their proposed research intentions — should be sent by (3 weeks after appearance) to:

The Assistant Director (Administration), MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH. Tel: Cambridge (0223) 248011. (2073)A

CSIRO
AUSTRALIA
ELECTRON MICROSCOPIST
(SENIOR RESEARCH SCIENTIST)
$A31,092-$A35,806
DIVISION OF FISHERIES RESEARCH
MARINE LABORATORIES
HOBART TAS

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Fisheries Research is a member of the Institute of Animal and Food Sciences.

GENERAL: The Division of Fisheries Research is a major component of the CSIRO Marine Laboratories, which is Australia's principal marine research institution. The Division currently employs about 70 scientists and 40 support staff based in laboratories in Sydney, Perth and Brisbane. The Sydney staff will be transferred in the latter half of 1984 to new laboratories being built at Hobart, Tasmania. The appointee will be located in the new laboratories which are expected to be completed about August 1984.

DUTIES: Responsible for management of the electron microscope laboratory consisting of scanning and transmission electron microscopes which will be used in research into physiology, cell biology and taxonomy of unicellular marine algae, ecology and taxonomy of protozoa and microzooplankton, reproductive physiology of fish, identification of fish eggs and larvae, and fish aging studies from growth increments (otoliths, scales, etc). The appointee must undertake a personal research program in one of the above fields with particular involvement in the micro-algal programs. Research into and development of improved techniques for preparation and examination of microalgal structure for taxonomic and biological investigations would be expected.

QUALIFICATIONS: A PhD degree or equivalent qualifications in biology with at least 6 years' post-doctoral research experience in electron microscopy. The appointee must have proven research achievement in biological electron microscopy, and extensive theoretical and practical knowledge of electron microscopes.

TENURE: Indefinite with Australian Government superannuation benefits available.

APPLICATIONS: Quoting reference No A3490, and giving full personal particulars, including details of qualifications and experience, copy of academic transcripts and the names of at least two professional referees, should be directed to:

The Acting Chief
CSIRO Division of Fisheries Research
PO Box 21
CRONULLA NSW 2230
AUSTRALIA

by June 22nd 1984.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W984)A

MEMBRANE STRUCTURE, FUNCTION & PATHOLOGY

The Faculty of Medicine, University of Alberta, invites applications for the positions of Research Scholar or Senior Scholar at the Assistant Professor ($27,720-$39,820) or Associate Professor ($35,400-$51,658) levels. The successful candidate(s) must have an MD, DDS and/or PhD degrees with at least two years of appropriate postdoctoral training and must have demonstrated outstanding potential for an independent research career in an area of the structure and function of biological membranes. These positions will be long term and will be funded through an external agency, but the incumbents will enjoy all the privileges of the respective University ranks. The granting of these awards is by competition and is made on the basis of excellence.

Candidates should send their curriculum vitae, outlines of proposed research, and the names of three references to Professor E.E. McCoy, Department of Pediatrics, University of Alberta, Edmonton, Alberta, Canada T6G 2G3.

The University of Alberta is an equal opportunity employer but in accordance with Canada Employment and Immigration regulations this advertisement is directed to Canadian citizens and permanent residents.

Closing date: August 1st, 1984. (NW535)A

RUHR – UNIVERSITÄT BOCHUM
Abteilung Für Biolgie

An der ABTEILUNG FÜR BIOLOGIE DER RUHR-UNIVERSITÄT BOCHUM ist der LEHRSTUHL FÜR ALIGEMEINE ZOOLOGIE wiederzubesetzen. Es wird erwartet, daß der Bewerber Lehrerfahrung in Allgemeiner Zoologie mit einer zukunftsweisenden Forschungsrichtung verbindet und sich an der Lehre im Grund- und Hauptstudium in angemessener Weise beteiligt. Eine Kooperation mit vorhandenen Forschungseinrichtungen ist erwünscht. Bewerbungen sind bis spätestens 15.6.1984 an den Dekan der Abteilung für Biologie der Ruhr-Universität Bochum, Postfach, D-4630 Bochum, zu richten.

(W981)A

RESEARCH DIRECTOR

INCELL, a research oriented molecular biology and fermentation technology organization, is located in the brewing and fermentation center of Milwaukee, Wisconsin, adjacent to the University of Wisconsin—Milwaukee and Marquette University.

Applications are now being accepted for the position of Research Director of our research laboratories from individuals with recent, broad experience in molecular biology with emphasis on molecular cloning, yeast genetics, and protein isolation and purification chemistry.

The individual selected will report directly to the President to help ensure the highest quality of research at INCELL and will contribute strongly to the success of the Company.

Responsibilities will include the design and establishment of new laboratory facilities, recruitment and supervision of qualified scientists and research assistants, consultation with engineering and production colleagues associated with our technology, and overall management and budgetary responsibility for INCELL's research and development program.

Minimum requirements for this position are a Ph.D. in genetics or molecular biology, preferably with emphasis in yeast genetics, and postdoctoral experience in the techniques of RNA and DNA manipulations. Applicants must be familiar with:

- Techniques of Molecular Cloning
- Classical Methods of Yeast Genetics
- Protein Biochemistry, including methods of analysis, isolation, and purification.
- Biochemistry and Genetics of protein secretion in microorganisms.
- Familiarity with nucleic acid sequencing and oligonucleotide synthesis methodologies.

A broad overview of molecular genetics with strong emphasis on yeast genetics and an interdisciplinary approach to problem solving are key qualifications for this position along with the capability of working in a relatively unstructured, fast moving entrepreneurial environment.

Qualified applicants for this executive level position are invited to submit, in confidence, their resumes together with publications and salary history to:

President, INCELL CORPORATION
Post Office Box 11596
Shorewood, Wisconsin 53211 U.S.A.

FAST MOVING, REAL WORLD ENTREPRENEURIAL ENVIRONMENT AND OPPORTUNITY—EQUITY PARTICIPATION.

(NW536)A

UNIVERSITY OF LONDON
BRITISH POSTGRADUATE MEDICAL FEDERATION
THE CARDIOTHORACIC INSTITUTE

Research position up to post doctoral level, for one year in the first instance, to join a group studying the regulation of collagen metabolism using in vitro (lung perfusion or tissue culture) methods. Experience in at least one of these techniques is essential.

Salary range £6,310-£7,190 plus £1,186 London Weighting depending on age and qualifications. For further details on the project telephone Dr G J Laurent 01-352 3378.

Applications to include curriculum vitae, list of publications and names of three referees should reach the Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP no later than 25 May 1984. (2059)A

UNIVERSITY OF WISCONSIN
Madison
POSTDOCTORAL RESEARCH

PhD in biochemistry or cell biology wanted to study ACTH control of cholesterol metabolism in adrenal cells at the molecular level. Project involves the mechanism of cholesterol metabolism at cytochrome P450 and of the actions of specific proteins that regulate cholesterol transport. Salary $14,000-16,000. Send curriculum vitae, publications, and names of three references to: Dr Colin R Jefcoate, Department of Pharmacology, University of Wisconsin Medical School, 1300 University Avenue, Madison, WI 53706.

(NW522)A

BIOANALYTICAL RESEARCH professional to conduct application research for separation and state o the art detection of trace amounts o biogenic compounds in a variety o biological tissue matrices, at Irvine CA. Must have MS in life science and a strong background in high per formance liquid chromatograph coupled with electrochemical an fluorometric detectors. Two year related research experience i industrial or academic setting knowledge of biochemical, neuro chemical applications and statistica analyses required. Ability to interac in an interdisciplinary environment i essential. Excellent oral and writte communication skills are a must Monthly salary $2,348. Send résum to the attention of Analytica Research, PO Box 19534, Irvine, C 92713. (NW531)A

ASSISTANTSHIPS

OXFORD UNIVERSITY
THEORETICAL CHEMISTRY DEPARTMENT
POST DOCTORAL RESEARCH ASSISTANTSHIPS

Applications are invited from theoretical chemists or physicists to work with Professor N H March on 'Electron density in large molecules of biological interest', the work being supported by the National Foundation for Cancer Research.

The appointments, for one year i the first instance, but expected to b renewed, are on the 1A Researc Support grade 1 with membership o USS. Starting salary is up to £8,975

Applications including a ful Curriculum Vitae and the names o two referees should be sent by 31 Ma 1984 to Professor N H March Department of Theoretica Chemistry, Oxford University 1 South Parks Road, Oxfor OX1 3TG. (1983)P

APPOINTMENTS WANTED

KEEN, BIOCHEMISTRY Graduate 2(i) with research experience in Molecular Biology, Protein Chemistry, GC/HPLC, seeks immediate position at any UK research establishment for post-graduate research. Box 2007, c/o Nature, 4 Little Essex Street, London WC2R 3LF (2007)B

APPOINTMENTS WANTED

advertise your qualities and qualif cations through the most influentia science weekly in the world at special reduced cost of *25p a word* (Personal Box Numbers £2). Advertisements must be pre-paid an sent to: Nature Classified (App Wtd), 4 Little Essex Street, Lond WC2R 3LF. (890)B

CONFERENCES and COURSES

UNIVERSITY OF ST ANDREWS
MSc Course in Radiation Biophysics

This taught course occupies a calendar year with a 6 month formal component and a 6 month research project.
The course is interdisciplinary and run jointly by the Departments of Anatomy and Experimental Pathology and Physics. It is structured to accommodate those qualified either in the physical sciences or the life sciences and caters for differing interests and career intentions of students. It is suitable, for example, for those considering careers in research with, and application and control of, radiation in science, industry, medicine and power production. Research Council scholarships (MRC) are available.
The course is that previously provided by the Department of Medical Biophysics at Dundee University.
Enquiries and applications to Dr A.C. Riches, Department of Anatomy and Experimental Pathology, University of St Andrews, St Andrews, Fife KY16 9TS. (2029)C

MISCELLANEOUS

RESEARCH VESSEL FOR ECONOMICAL WORLD WIDE CHARTER

A 55'6" × 19' crabber/seiner desig of Ed Monk with a range of 6,00 miles at eight knots. Party of six, ex perienced crew, extensive electronic including SATNAV.

Spice Island Traders, One Bosto Place, Boston, MA 02108 (617 723-1955. (NW750)J

Please mention
nature
when replying to these advertisements

FELLOWSHIPS

UNIVERSITY OF ASTON IN BIRMINGHAM
DEPARTMENT OF PHARMACY

The Department is in the process of establishing a Pharmaceutical and Biomedical Sciences Research Institute to co-ordinate its research activities and to concentrate its resources in areas which are innovative and commercially relevant. Applications are now invited for the following posts:

RESEARCH FELLOWSHIP IN PHARMACEUTICS (Ref 607/6)

This one-year post (available immediately) would interest a recent PhD graduate in pharmaceutical or chemical sciences with interests in the physico-chemical aspects of drugs and drug-delivery. The successful candidate will be required to contribute to the undergraduate course in the physico-chemical aspects of pharmacy and the design of drug-delivery systems and to undertake research within the framework of the Drug Development Group with specific interests in drug discovery, drug development, and drug dosage-form design.

Commencing salary will be within the range £7,190 to £9,425 per annum (Research Range 1A).

Application forms and further particulars may be obtained from the Staff Officer (quoting Ref 607/6), the University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET (Tel 021-359 3611 ext 4564). Closing date 8th June 1984.

RESEARCH STUDENTSHIPS

A number of studentships (from Research Councils, Industry and other sources) are available for full-time study for a Higher Degree commencing 1 October 1984. Graduates with a First Class or a 2(i) Honours Degree in the pharmaceutical, life or physico-chemical sciences, or students expecting to graduate this summer, are invited to apply for posts within the following research groups.

Cancer Research Campaign Experimental Chemotherapy Group
Drug Development Group
Drug Mechanisms Group
Medical Microbiology Group
MRC Mechanisms of Drug Toxicity Group
Pharmacy Practice Group
Rheumatism and Arthritis Group

Applicants seeking further information on the research projects available within these Groups are invited to write in the first instance to: Dr J A Hickman, Department of Pharmacy, University of Aston in Birmingham, enclosing a curriculum vitae and identifying their particular research interests. (2052)E

E M B O
EUROPEAN MOLECULAR BIOLOGY ORGANIZATION
SHORT TERM FELLOWSHIPS in molecular biology

The European Molecular Biology Organisation awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in extremely exceptional circumstances.

Application forms and further details may be obtained from Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F.R.G. (W818)E

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP IN BIOCHEMISTRY (DRUG METABOLISM)

Applications are invited for the post of Research Fellow to pursue a programme of research under the supervision of Dr L A Griffiths into the metabolism of O-heterocyclic drugs. Previous experience of drug metabolism research is desirable but not essential but the candidate should possess postgraduate experience in radiochemical techniques and in either h.p.l.c. or g.l.c. procedures.

Post tenable for up to two years (renewable at end of first year). Starting date between 4 June and 2 October 1984.

Salary on the Research Fellow 1A scale £7,190-£11,615 plus superannuation, according to qualifications and experience.

For further particulars phone 021-472 1301 ext 2559, quoting reference B5.

No formal application form. Three copies of application, including full Curriculum Vitae, naming three referees and indicating date of availability, to Assistant Registrar, (Science and Engineering), P O Box 363, Birmingham B15 2TT, by 25 May 1984. (2006)E

UNIVERSITY OF SASKATCHEWAN
Drug Metabolism and Drug Disposition Research Group
COLLEGE OF PHARMACY

POSTDOCTORAL FELLOWSHIP

Applications are invited for the above position to work on the synthesis and metabolic application of deuterium labelled phenothiazine antipsychotic agents.

The position is tenable immediately and is for two years. Initial salary depends upon experience. Applications, including Curriculum Vitae and the names of three referees, should be sent to: Dr E M Hawes, College of Pharmacy, University of Saskatchewan, Sasktoon, Saskatchewan, Canada S7N 0W0 (306) 343-4788. (NW526)E

MULTIPLE SCLEROSIS SOCIETY RESEARCH TRAINING FELLOWSHIP

The Multiple Sclerosis Society requires a postdoctoral Cell Biologist to work for 3 years in the first instance on the nature and function of the cellular infiltrate in the brain in models of demyelinating disease.

The post is tenable in the Department of Pathology of the Royal College of Surgeons of England. Salary on University of London scales according to age and experience.

It is hoped the appointment would be made with effect from 1st September, 1984.

Applications, which should be made by 18th June to be sent to: Professor J. L. Turk, Department of Pathology, Institute of Basic Medical Sciences, Royal College of Surgeons, Lincoln's Inn Fields, London WC2A 3PN. (2072)E

MS MULTIPLE SCLEROSIS

SEMINARS and SYMPOSIA

INTERNATIONAL SYMPOSIUM
on
PAN-AFRICAN CRUSTAL EVOLUTION IN ARABIA AND NORTHEAST AFRICA

The second IGCP-164 symposium will be held on 1st — 10th February 1985 in the Faculty of Earth Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. Field excursions are planned after symposium. Papers are invited.

For details, please write to Dr Ahmed Al-Shanti, Project Leader, IGCP-164, PO Box 1744, Jeddah 21441, Saudi Arabia. (W994)M

THE 6TH INTERNATIONAL SYMPOSIUM
on
NITROGEN FIXATION
will be held at
OREGON STATE UNIVERSITY
IN CORVALLIS
August 4-10, 1985.

The symposium will include chemistry, biochemistry, physiology, microbiology, genetics, ecology and applied aspects of the field. Lectures, discussions, poster sessions and excursions are planned. Those desiring to receive the first circular should contact, 6th International Symposium on Nitrogen Fixation, Agricultural Experiment Station, Oregon State University, Corvallis, OR 97331, USA. (NW533)M

STUDENTSHIPS

UNIVERSITY OF OXFORD
Nuffield Department of Orthopaedic Surgery
MRC Bone Research Laboratory
Applications are invited for a

MRC Research Studentship

to join an active research team for work on the differentiation of bone-forming cells in normal and pathological conditions with particular reference to the characterisation of cell-specific markers by using monoclonal antibody techniques. Applicants should have, or expect to obtain, a first or upper second class honours degree in biochemistry and will be expected to register for a D.Phil degree.
The names and addresses of two referees and a complete curriculum vitae should be sent as soon as possible to Dr. J.T. Triffitt or Dr. Maureen Owen, MRC Bone Research Laboratory, Nuffield Orthopaedic Centre, Oxford OX3 7LD. (2047)F

Continued on pages 16 & 17

STUDENTSHIPS continued

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Studentships for postgraduate conversion courses for up to one year.

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IT covers information acquisition, handling and use involving the latest technologies in microelectronics, computing and telecommunications.

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SERC's one-year studentships on postgraduate conversion courses are part of an IT initiative promoted by the Department of Education and Science.

For details about SERC studentships contact:

Information Technology Studentships,
Science and Engineering Research Council,
North Star Avenue,
SWINDON SN2 1ET
Telephone: 0793 26222 Ext 2137

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(2067)F

UNIVERSITY OF LONDON
Institute of Psychiatry

DEPARTMENT OF PSYCHOLOGY

RESEARCH STUDENSHIP

The Department invites applications for an MRC postgraduate research studentship. The successful applicant will register for a London PhD and can choose from a variety of projects related to current research in the Department. This ranges from studies of neurophysiological and neurochemical mechanisms in the control of behaviour in the rat to work on behaviour and personality in both normal and psychiatrically disturbed human subjects.

Candidates should hold or expect to receive first or upper second class honours degrees in psychology or a related discipline in the life or social sciences.

Applicants should write to Professor J A Gray, Department of Psychology, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, giving details of their curriculum vitae, the names of two referees and an indication of their preferred field(s) of research. Closing date: 31 May 1984. (2058)F

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF ZOOLOGY

SERC STUDENTSHIP IN CEPHALOPOD NEUROBIOLOGY

Applications are invited for the above studentship, tenable for 3 years commencing October 1984. Candidates will be expected to register for a PhD degree and must have a good honours degree in Anatomy, Zoology, Physiology or a related subject.

The research will be in the area of cephalopod neurobiology, in particular the central control and regulation of colour patterning. The main emphasis will be on applying and developing modern anatomical techniques.

Applicants should write, giving the names and addresses of two referees and full details of their academic career to date, to the project supervisor: Dr J B Messenger, Department of Zoology, University of Sheffield, Western Bank, Sheffield S10 2TN. Quote ref: R 81/G. (2021)F

UNIVERSITY OF SOUTHAMPTON

SCHOOL OF BIOCHEMICAL & PHYSIOLOGICAL SCIENCES

SERC CASE STUDENTSHIP

An SERC Case studentship is tenable from 1st October 1984 for a three year period in the Neurophysiology Department of the School in conjunction with Roussel Laboratories, Swindon.

The project will involve studies on central GABA receptors in the rat. The following methods will be used: electrophysiological studies using in vitro slice preparations and release studies using in vitro slice preparations and release studies from cortical and cerebellar slices. A knowledge of electrophysiological recording methods would be an advantage.

Applicants, who must hold or expected to obtain a I or II (1) class Honours degree in Physiology, Pharmacology, Biology or a related subject, will be expected to register for a PhD degree. Applications, including curriculum vitae and the names of two referees, to Dr R J Walker, School of Biochemical & Physiological Sciences, University of Southampton, Southampton SO9 3TU. Tel: 0703-559122 ext 8343 or 8341. (2011)F

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF MEDICAL PHYSICS AND CLINICAL ENGINEERING

RESEARCH STUDENTSHIP

An SERC studentship is available to pursue research in an area of Medical Physics leading to a PhD. The successful applicant will be expected to have obtained at least a 2.1 in physics or electronic engineering. Applications with a CV should be made to Professor M M Black at the above department; Royal Hallamshire Hospital, Sheffield S10 2JF from whom further details may be obtained. Tel: 0742 26484 ext 2712 or Dr D C Barber. 2688. Closing date 11 June 1984. Quote ref: R85/9. (2066)F

UNIVERSITY OF MANCHESTER

DEPARTMENT OF ZOOLOGY

NERC STUDENTSHIP

Applications are invited for a research studentship to investigate ecological and genetic diversity using computer modelling techniques. Candidates should have, or expect to obtain in 1984, a first or upper second class honours degree in a relevant biological subject or in mathematics or physics.

Further details can be obtained from Dr L M Cook, Department of Zoology, University of Manchester, Manchester M13 9PL, to whom letters of application including a curriculum vitae and the names of two academic referees should be sent as soon as possible. (2020)F

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PATHOLOGY

PhD STUDENTSHIP

Applications are invited for research studentship (supported b the British Heart Foundatio tenable for 3 years commencin October 1984 to study the role c vitamin B6 deficiency in the develo ment of atherosclerosis. The wor involves investigation of the mode c action of lysine oxidase, the copp and pyridoxal-dependent enzym responsible for the cross-linking c collagen and of elastin; it involv animal experimentation as well a biochemistry. A background in bi chemistry and biology is essential.

Candidates should hold, or expe to obtain a First or Upper Secon Class Honours Degree in Bi chemistry. Applicants should app in writing including their curriculu vitae and the names of two academ referees to Dr C I Leven Department of Pathology, Unive sity of Cambridge, Tennis Cou Road, Cambridge CB2 1QP. (1980)F

HERIOT-WATT UNIVERSITY

CASE STUDENTSHIP IN OFFSHORE ENGINEERIN

Applications are invited from Engi eering or Science Graduates for immediately available resear studentship. The project is concern with the effect of fouling and surfa roughness on the wave and curre loading on marine structures.

Support is for a period of thr years and the successful applica would be expected to register for higher degree. Applicants shou hold a first class or upper second cla honours degree.

For further particulars plea contact Dr I Grant, Department Offshore Engineering, Heriot-W University, Edinburgh. Telepho 031-449 5111 ext 2319. (2025)F

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF ZOOLOGY

SERC CASE STUDENTSHI

Trematode larvae and the sn immune response.

Applications are invited fr recent graduates or those about graduate in a Biological Science fo CASE studentship leading to a Ph The project in collaboration with V R Southgate (British Muse Natural History) will investigatge t cellular basis of host-parasite int actions between the blood flu Schistosoma and the snail Bulin both in vivo and in vitro.

Applications with a curricul vitae and the names of two refer should be sent to Mr T M Prest Zoology Department, UCL, Gow Street, London WC1E 6BT fr whom further information may obtained. (2012)F

STUDENTSHIPS continued

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF BOTANY AND MICROBIOLOGY

NERC STUDENTSHIP IN MICROBIAL ECOLOGY

The NERC have awarded a studentship to the Department for studies of ColV$^+$ enterobacteria. Such organisms occur in sewage and contaminated water and are important in Public Health because ColV frequently increases bacterial pathogenicity. The aims of the project are to examine natural occurrence and location of ColV$^+$ bacteria and to isolate new ColV$^+$ strains. These will then be compared with established ColV$^+$ strains with respect to plasmid DNA content, envelope protein composition, and other plasmid specified properties. It is then proposed to examine behaviour of ColV$^+$ organisms in the environment studying (i) ColV plasmid stability, (ii) ColV plasmid transfer, (iii) competition between ColV$^+$ and ColV$^-$ organisms (especially at low iron concentrations as some ColV plasmids specify an iron chelation mechanism), (iv) survival of ColV$^+$ organisms compared to ColV$^-$ ones.

The studentship would be suitable for a candidate studying Microbiology or a related Biological subject and who has already obtained or hopes to obtain in June 1984) an honours degree with at least upper second class honours. For further details, apply to Dr R J Rowbury Department of Botany and Microbiology, University College London, Gower Street, London WC1E 6BT. (2033)F

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

BIOPHYSICS GROUP

RESEARCH COUNCIL STUDENTSHIP

Applications are invited for a Research Council Studentship for work in the Biophysics Group, for research on one of the following topics:

— Crystallography of enzymes, including molecules created by protein engineering:

— Molecular mechanisms of general anaesthesia:

— Three-dimensional structure of vertebrate muscle:

— Effects of neuroactive substances on retinal neurones:

— Kinase control of microtubule assembly:

— Design of inhibitors of angiotensin converting enzyme (CASE).

Applicants must expect or possess a good honours degree in physics, biochemistry or another relevant subject.

Please apply directly to Prof. D.M. Glow, Blackett Laboratory, Imperial College, London SW7 2BZ, with a brief C.V., naming two referees. (2036)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BOTANTY

SERC RESEARCH STUDENTSHIPS

The following SERC research studentships are available within the Department of Botany tenable from October 1st, 1984. Two awards are available.

1. Control of secondary product formation in plant tissue cultures (Dr H A Collin).

2. Physiology and biochemistry of protoplasts isolated from Phytophthora infestans. (Dr S Isaac and Dr A M Mortimer).

3. Polymeric soil conditioners as aids to vegetation establishment on difficult soils (Dr M S Johnson).

Further details of these studentships are available from Professor A D Bradshaw, Department of Botany, University of Liverpool, PO Box 147, Liverpool L69 3BX. Applicants should have or be expecting at least a 2:1 Honours Degree in an appropriate biological subject. The Department of Botany has a very active research school with excellent facilities, concerned with pure and applied aspects of botany.

Applications should be made as soon as possible and at least by June 4th to The Registrar, University of Liverpool, PO Box 147, Liverpool L69 3BX. They should include details of academic experience and the names of two referees.

Quote Ref: RV/508/N. (2027)F

UNIVERSITY OF CAMBRIDGE

THE BIOTECHNOLOGY CENTRE

SERC STUDENTSHIPS IN BIOTECHNOLOGY

Applications are invited from graduates in appropriate disciplines for the following research studentships tenable for three years in the newly established Biotechnology Centre and involving the development of novel biological sensors for application in clinical diagnostics:

1. Fibre Optic Biosensors, SERC Quota Award, Tenable from 1st October 1984.

2. Immunologically-sensitised Field Effect Transistors (Immuno-FETs), SERC Replacement Quota Award, Tenable from July 1984.

3. Integrated Optical Biosensors, SERC CASE Studentship, Cooperating Body: Plessey Research (Caswell) Ltd, Industrial Supervisor: Dr W J Stewart, Tenable from 1st October 1984.

Applicants must hold, or expect to gain, a first or upper second class honours degree and should send a curriculum vitae together with the names and addresses of two referees and indicating which post is being applied for, to: Dr C R Lowe, The Biotechnology Centre, University of Cambridge, Downing Street, Cambridge CB2 3EF. Tel: (0223) 60153. (2050)F

NATURAL ENVIRONMENT RESEARCH COUNCIL

CASE RESEARCH STUDENTSHIPS WITH BRITISH ANTARCTIC SURVEY

Applications are invited for seven NERC CASE studentships awarded under the Antarctic Research Special Topic programme and leading to a PhD degree:

Life Sciences

Predation in a simple terrestrial community (Dr M B Usher, Department of Biology, University of York, York YO1 5DD).

Comparative physiological adaptation of selected Antarctic microbial communities to low temperature (Dr D B Nedwell, Department of Biology, University of Essex, Colchester CO4 3SQ).

Autecological study of a planktonic predatory amphipod *Parathemisto gaudichaudii* in Antarctic waters (Dr M Sheader, Department of Oceanography, University of Southampton, Southampton SO9 5NH).

Earth Sciences

Global biostratigraphy, biogeography and evolution of the early Cretaceous bivalve *Aucellina* (Dr P F Rawson, Department of Geology, University College London, Gower Street, London WC1E 6BT).

Atmospheric Sciences

Halley-St Anthony ULF-VHF conjugate studies (Dr D Orr, Department of Physics, University of York, York YO1 5DD).

Radiometric observations of ocean and atmosphere by ship and satellite borne sensors (Dr E J Williamson, Department of Atmospheric Physics, University of Oxford, Clarendon Laboratory, Parks Road, Oxford OX1 3PU).

Generation and behaviour of internal gravity waves in the atmospheric boundary layer (Dr J Brindley and Dr S D Mobbs, Department of Applied Mathematics, University of Leeds, Leeds LS2 9JT).

Studentships are tenable for three years from 1 October 1984 and applicants should hold or expect to obtain at least an upper 2nd class honours degree. Further details of projects available from the university supervisors to whom applications should be made by 29 June. (2071)F

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

SERC RESEARCH STUDENTSHIP

Applications are invited for a CASE Studentship starting date 1 October 1984. It is tenable for three years and, subject to satisfactory progress, will lead to a PhD. Applications are invited from students expecting at least an upper second class Honours degree in an appropriate subject.

The work involves strain improvement in the yeast Saccharomyces cerevisiae by manipulating genes involved in ammonium assimilation. Conventional yeast genetics as well as recombinant DNA technology will be used. The collaborating body is the Distillers Company Food Group.

Applications, with the names of two referees, should be sent to The Secretary, Department of Biochemistry and Microbiology, University of St Andrews, St Andrews, Fife. (2069)F

ROYAL POSTGRADUATE MEDICAL SCHOOL (University of London)

DEPARTMENT OF MEDICINE (MRC CLINICAL IMMUNOLOGY RESEARCH GROUP)

MRC STUDENTSHIP

Applications are invited for a three year MRC research studentship tenable from October 1984.

The project will be in the area of the molecular basis for the effects of persistent virus infection on the immune response, working with human cytomegalovirus.

Applicants should send a full CV to the Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London, W12 OHS, quoting reference number 2/MRC.

Informal enquiries to Dr J G P Sissons on 01-743 2030 ext 345.

Closing date: 7 June 1984. (2038)F

UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY

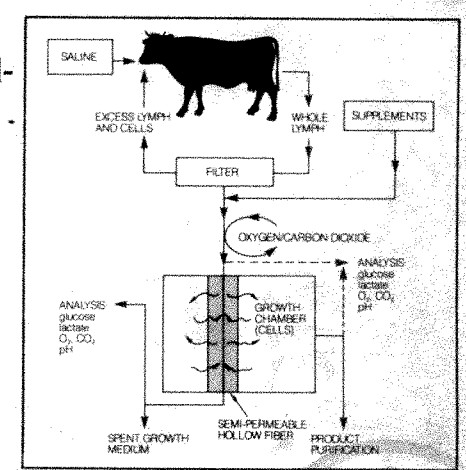
SERC-CASE STUDENTSHIP

Applications are invited from graduates who have or expect to receive a first or upper second class degree in Biochemistry for a 3 year CASE award to work on the structure and activity of the membrane receptor for the anti-asthmatic drug, sodium cromoglycate. The project is a collaborative one between Dr J Findlay at the University of Leeds and Fisons plc, manufacturers of the drug. A cv together with the names of 2 referees should be sent as soon as possible to Dr J Findlay, Department of Biochemistry, University of Leeds, LS2 9JT. (2042)F

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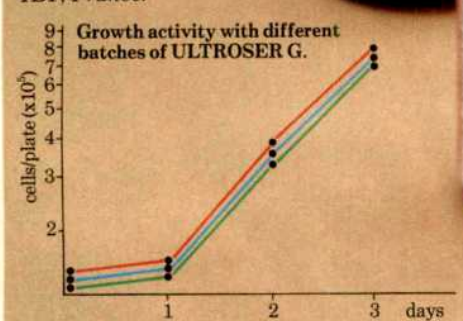
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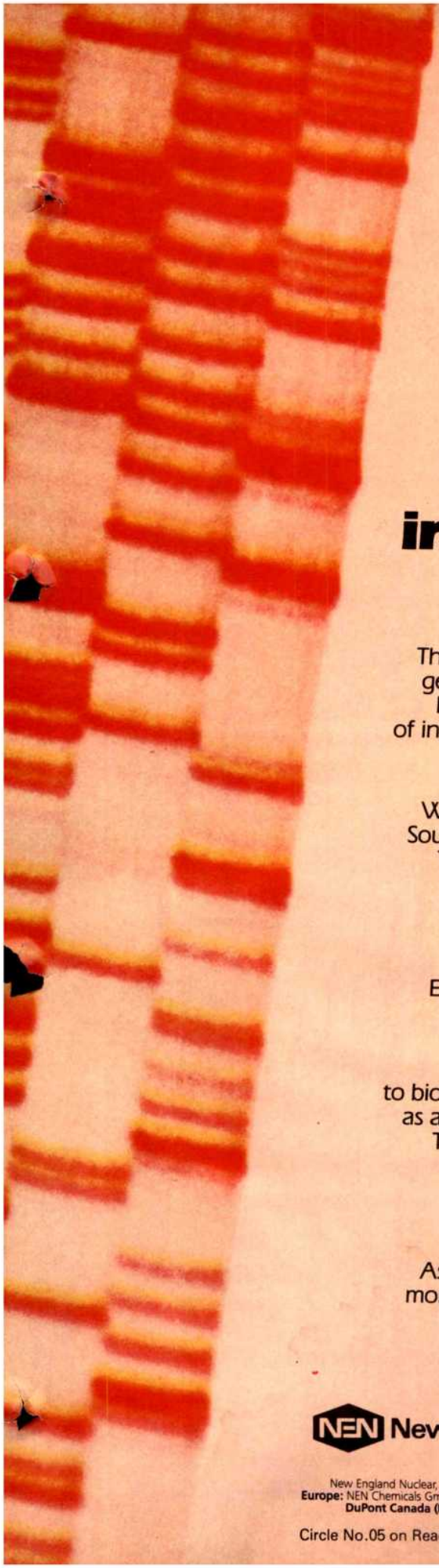
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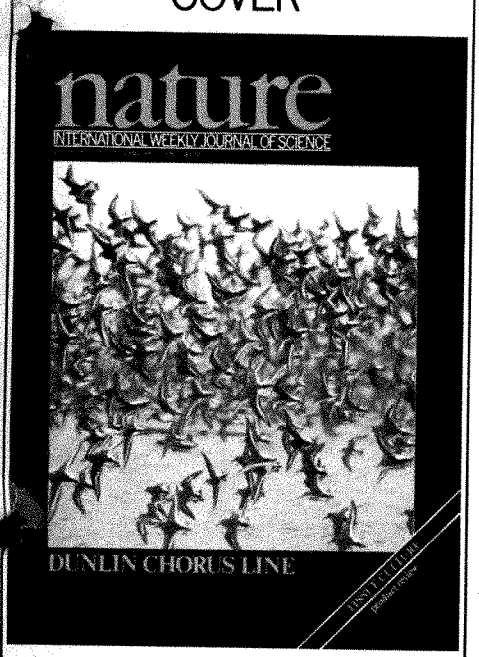
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COVER



Abrupt, coordinated changes of direction in a flock of birds (like the dunlins shown here) seem to be initiated by any bird executing a maneouvre towards the flock. The "manoeuvre wave" appears to be propagated in much the same way as occurs in a human chorus line (see p. 344).

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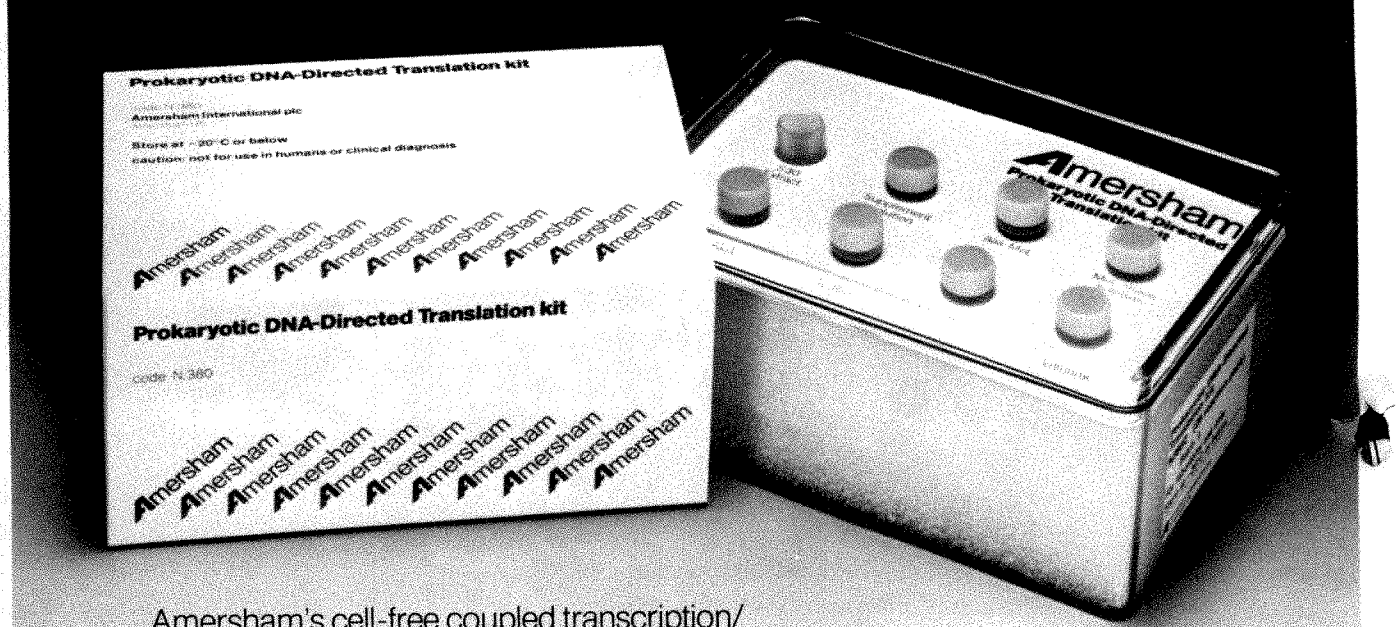
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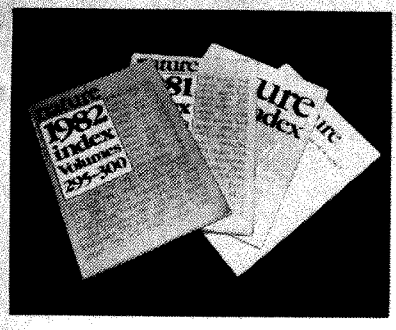
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- Running squid sodium pump backwards
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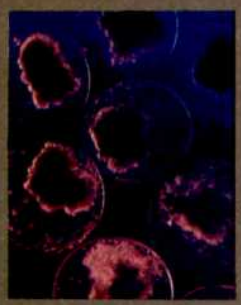
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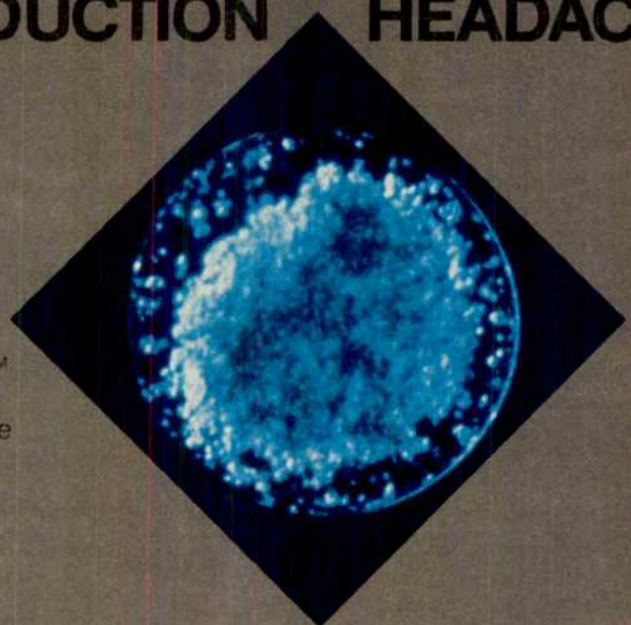
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Center: high levels of mouse antibody are produced within each microcapsule

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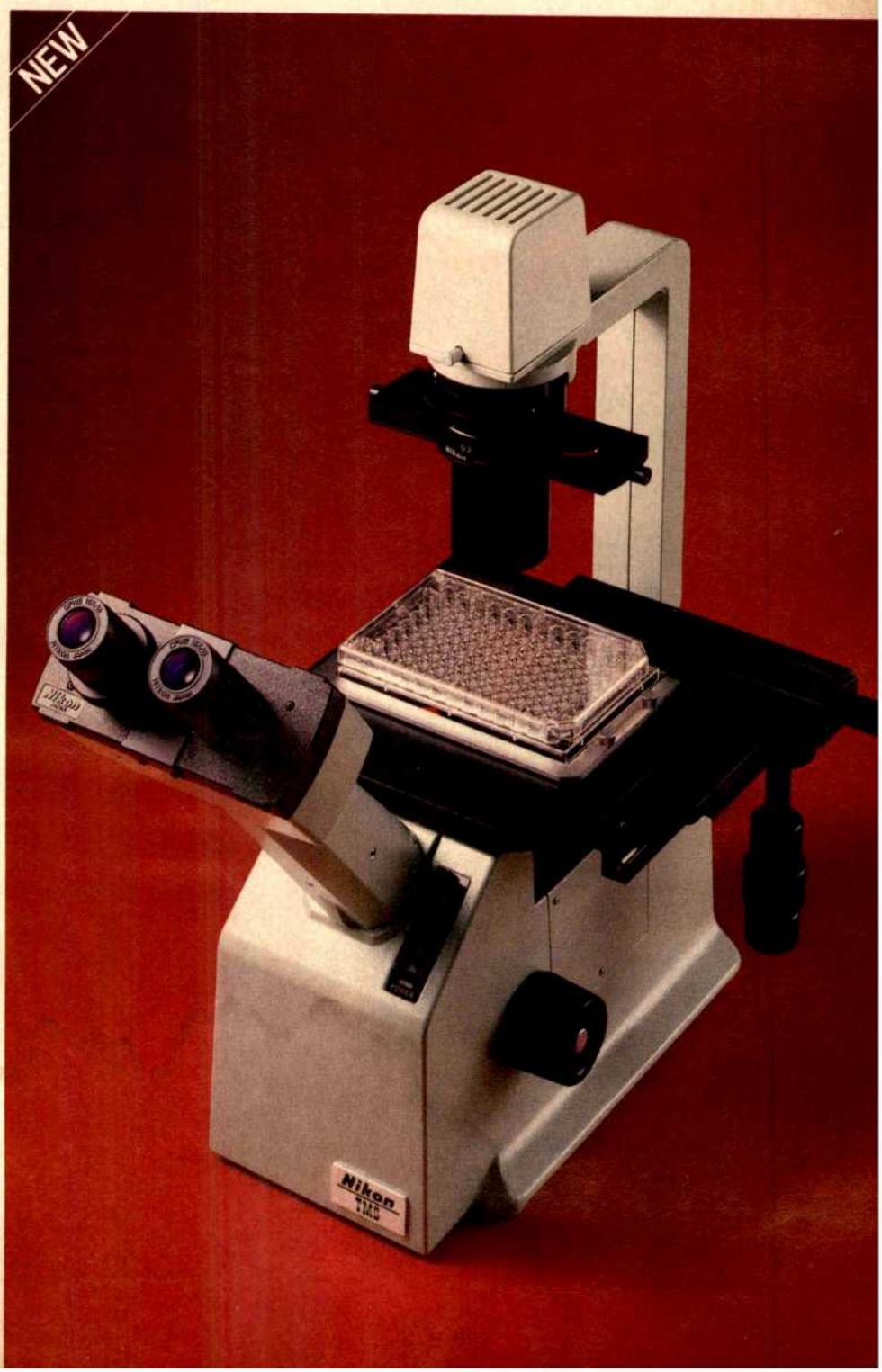
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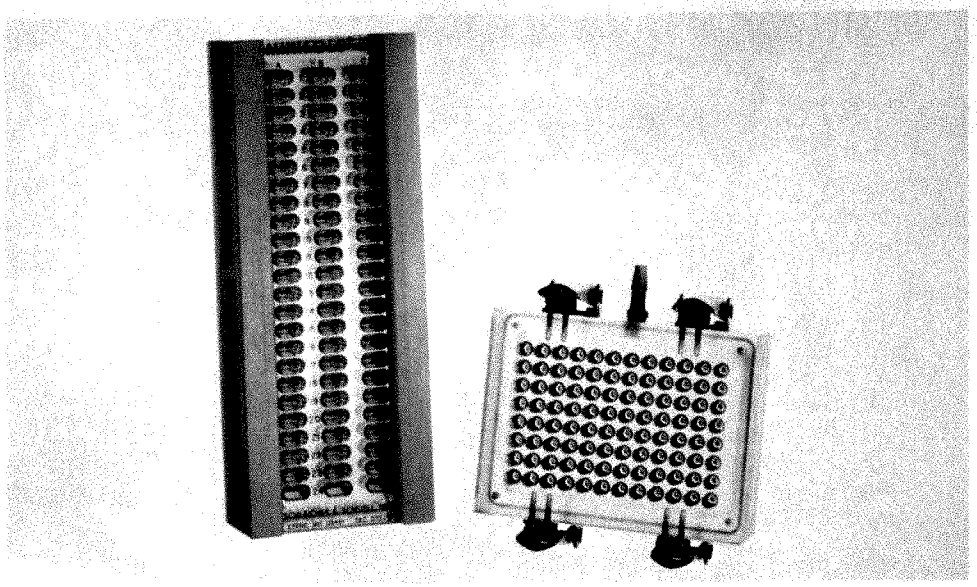
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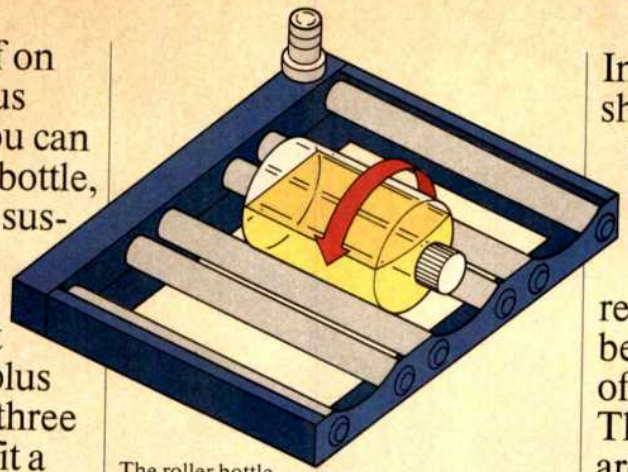
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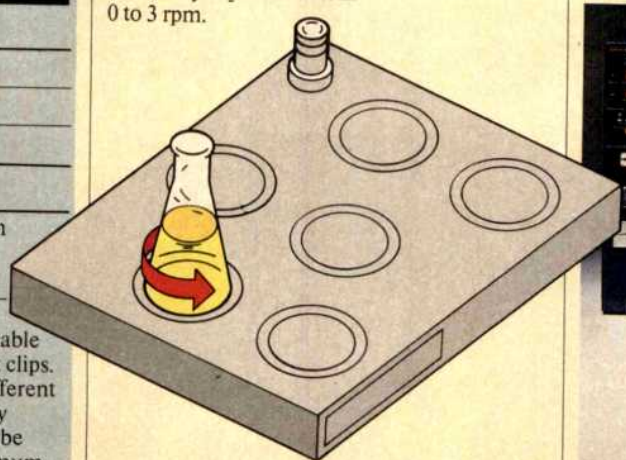
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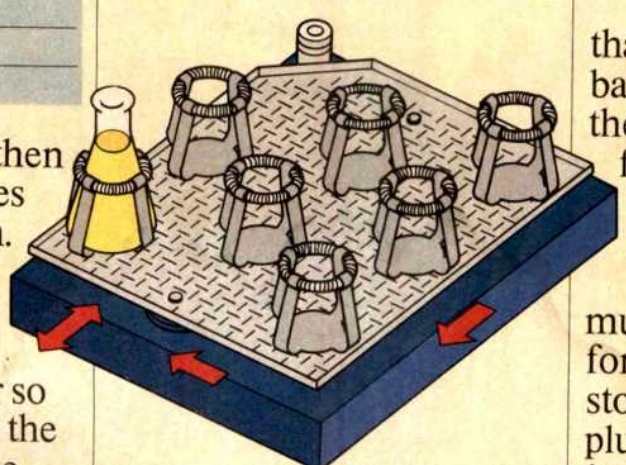
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Recent developments in molecular biology, many of which have been described by their authors in *Nature*, are helping to clarify the causes of cancer. With each passing week, more and more people consider that a full understanding of cancer cannot be long delayed. But the implications for physicians and their patients are still entirely unclear.

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THE MOLECULAR BIOLOGY OF

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September 10-12, 1984
Boston Park Plaza Hotel

Daily Themes:

DAY 1 - ONCOGENES,
DAY 2 - THE CANCER CELL,
DAY 3 - PROSPECTS/PROBLEMS.

DAY 1

MONDAY, 10 September
Exhibition Viewing: 8:30am— 6:30pm
Recruitment Center: 9:30am— 6:00pm

ONCOGENES AND GROWTH FACTORS — WHAT THEY ARE AND WHAT THEY DO

9:00— 9:15	Chairman
9:15—10:00	Oncogenes *R. Weinberg (MIT)*
10:00—10:45	Growth factors *M. Sporn (NIH)*
11:15—12:00	HTLV *R. Gallo (NIH)*
12:00—12:30	Clinician's View *S. Broder (NIH)*
2:00— 4:00	Roundtables with morning speakers
4:30— 5:30	The Cancer Cell *J. Cairns (Harvard)*

DAY 2

TUESDAY, 11 September
Exhibition Viewing: 8:30am— 6:30pm
Recruitment Center: 8:30am— 6:00pm

THE CANCER CELL — HEREDITARY CANCER, TUMOR PROMOTERS AND CARCINOGENESIS

9:00— 9:15	Chairman
9:15—10:00	Gene Regulation *P. Leder (Harvard)*
10:00—10:45	Hereditary cancer *R. White (Utah)*
11:15—12:00	Carcinogens and promoters *I.B. Weinstein (Columbia)*
12:00—12:30	Clinician's View *F. Gilbert (Mt. Sinai)*
2:00— 4:00	Roundtables with morning speakers
4:30— 5:30	NCI program

DAY 3

WEDNESDAY, 12 September
Exhibition Viewing: 8:30am— 3:00pm
Recruitment Center: 8:30am— 3:00pm

PROSPECTS AND PROBLEMS— DRUG RESISTANCE, MONOCLONAL ANTIBODIES AND NEW DRUGS

9:00— 9:15	Chairman
9:15—10:00	Diagnostics and therapeutics *G. Todaro (Oncogen)*
10:00—10:45	Immunotoxins *J. Uhr (Texas)*
11:15—12:00	Drug resistance *A. Varshavsky (MIT)*
12:00—12:30	Clinician's View *J. Gutterman (Houston)*
2:00— 4:00	Roundtables with morning speakers
4:00— 4:45	Prospects
4:45	Closing remarks

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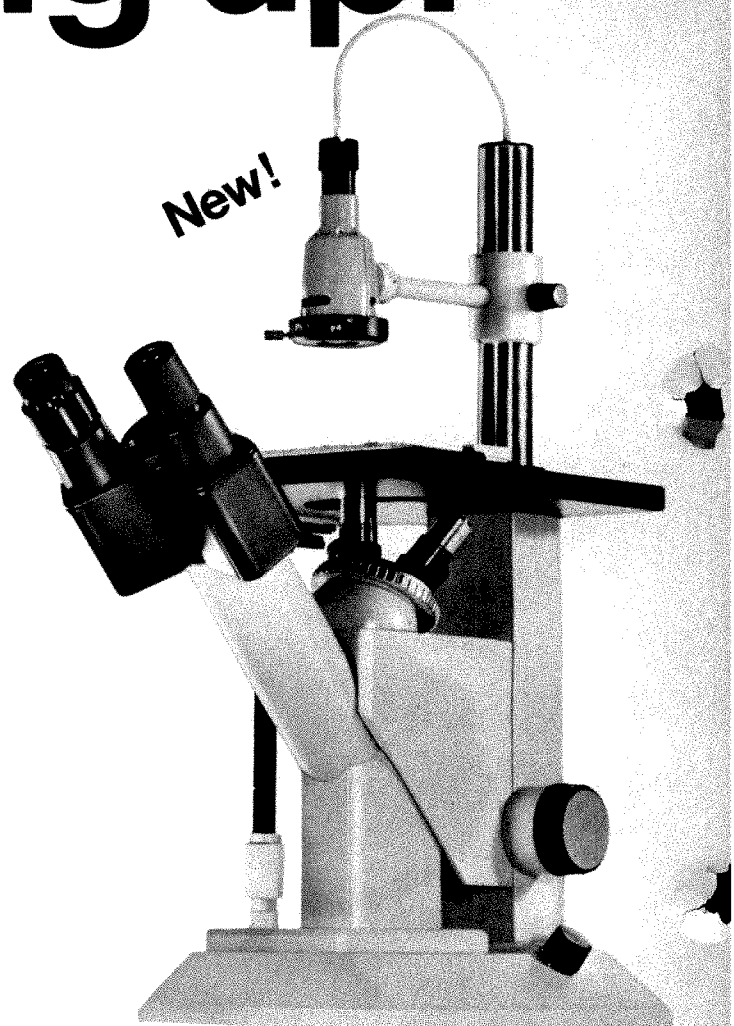
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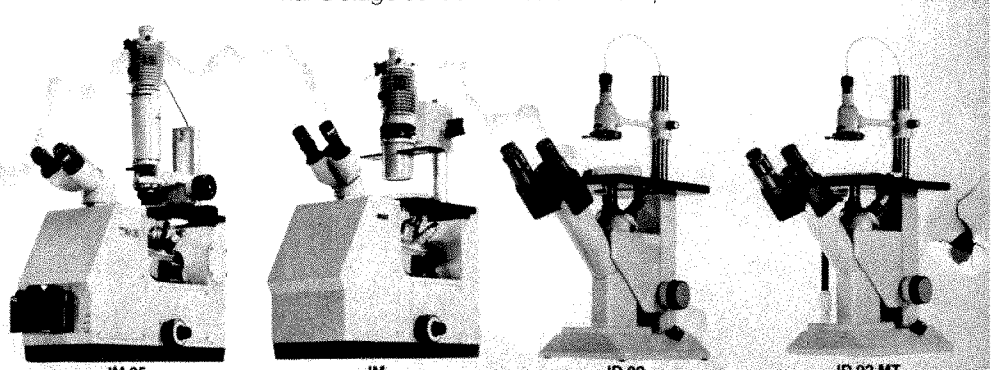
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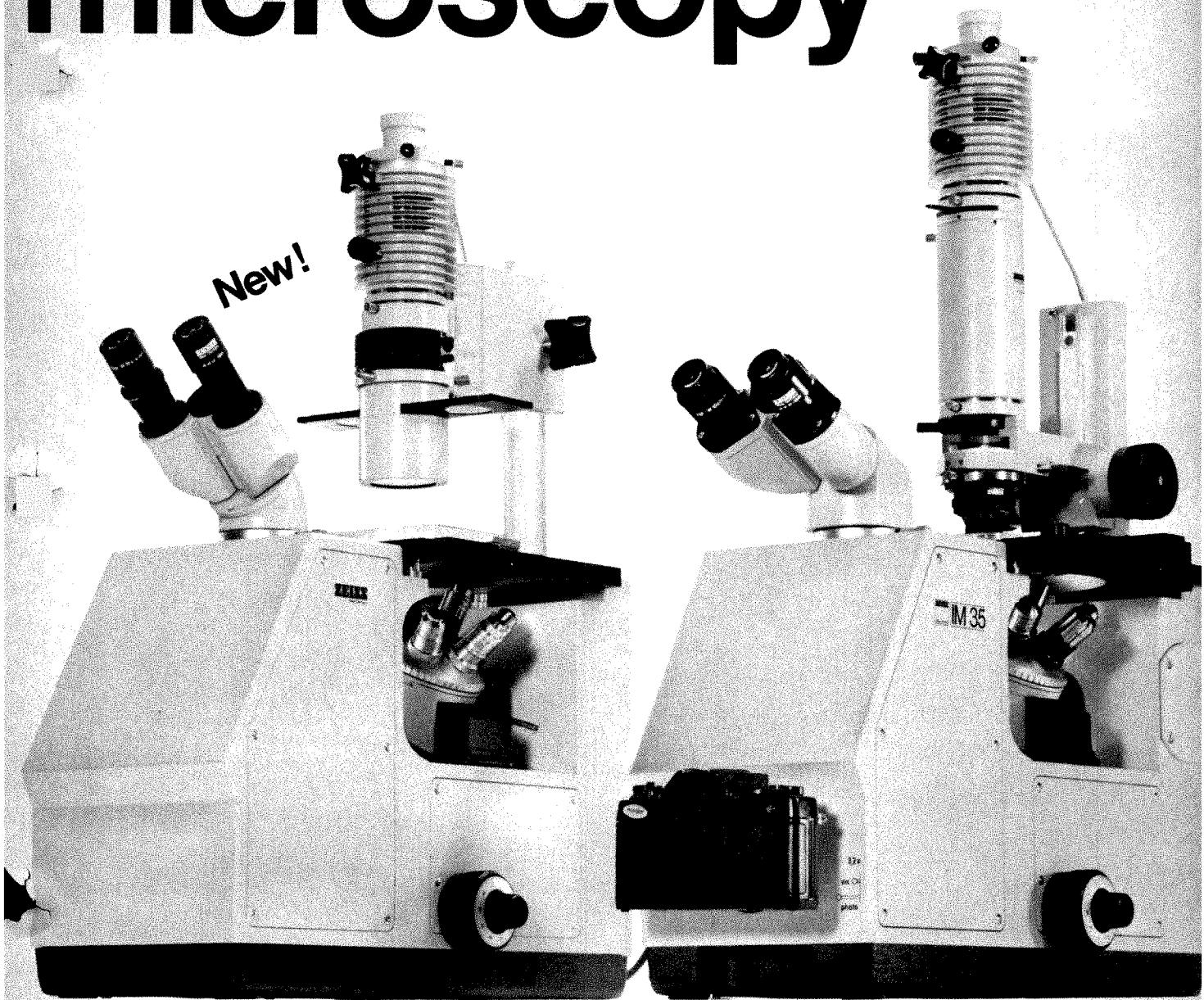
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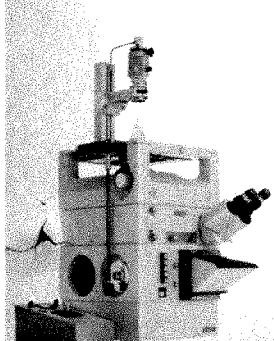


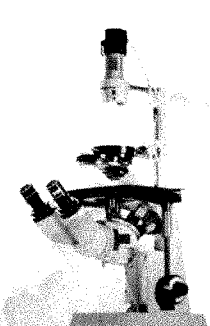
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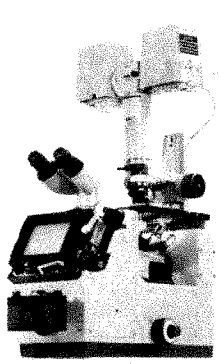
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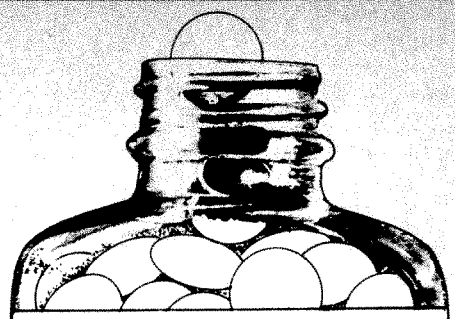
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REGULATION AND RESTRAINT IN CONTEMPORARY MEDICINE IN THE UK AND USA

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Presenting the edited proceedings of an Anglo-American conference, held in October 1981, this book examines in detail the increasing regulatory pressures placed upon medical practice and research both from inside and outside of the profession. These pressures have led to a conflicting array of self-imposed as well as legally imposed constraints, and the contributors discuss here their desirability for both the patient and the profession.

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Medicine in a regulated world
Session II
Factors which can harm the patient
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Constraints in General and Consultant Practice
Session V
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nature

Prejudice and academic tenure

The British Government plans to intervene directly in the ancient argument about the terms on which university teachers are appointed. It may irreparably damage the universities in the process.

BRITISH governments, even those like the present which profess that government should be unobtrusive, are chronically inclined to centralism. That is the only charitable reading of the decision (see *Nature* 17 May, p.197) that the government intends to intervene directly on the issue of academic tenure in British universities. The plan, which will no doubt be legitimized by the government's huge majority in the House of Commons, is that a group of unnamed statutory commissioners should be given the power to amend the charters which allow British universities to operate as such. The excuse, according to the Secretary of State for Education and Science, Sir Keith Joseph, is that universities have been slow, these past two years, voluntarily to extinguish academic tenure and are probably constitutionally incapable of doing so. The truth is that the present government is deeply offended by the common (but not universal) clause in British academics' contracts of employment that appointments will continue until "normal retirement age", and is impatient to see these arrangements swept away. By proposing to intervene directly, however, the government has confirmed its reputation as one that lets prejudice dictate policy.

The case for academic tenure is widely misunderstood, but derives from the doctrine that universities are self-governing communities of scholars within which people may (and will) disagree on questions as different as the allocation of car-parking space and the best route to the quantization of the gravitational field. The past six centuries have provided ample evidence that universities are most creative when individuals are able to follow lines of inquiry which their immediate colleagues may consider unproductive, and which fall outside public definitions, by governments and others, of social needs. Both in research and teaching, the indispensable social function of universities is the level-headed iconoclasm of which they are the only continuing source. Unfortunately for the government, this requires that academics should enjoy a measure of intellectual independence even from those who are titularly superiors. It would be (and when attempted is) disastrous if heads of academic departments sought to organize their bailiwicks' research as if it were an industrial research programme directed at a well-defined objective. In industry, it is proper that somebody unwilling to conform with the overall plan should be asked to find a job elsewhere. Human nature being what it is, heads of university departments are not always free from the temptation to ponder the advantages of such arrangements. The freedom to dissent even from close colleagues' opinions is what the tenure system was intended to preserve.

Security

But why should academics be insulated from the market forces to which all other people are exposed? That is what the British Government has been asking these past five years. The question is plausible enough, but begs another provoked by the way in which the British Government itself has been rigging the market in which British universities now operate. The decree more than three years ago that student numbers must be limited, and the foolish translation of this demand into student quotas for every British university by the University Grants Committee, means that British academics are unable to strive to ensure their own security by better satisfying the demand for higher education. Special public sponsorship, more recently, for the provision of higher education in fields such as information technology, while generally welcomed by hard-pressed universities, is another sign that the academic market is not a genuine interaction between self-governing communities of academics and would-be students but a process in which often arbitrary external influences play an important part. But unpredictable changes in the environment in which academics must work are not always as remote. Last week's report from the science board of the Science and Engineering Research Council (see p.297), the chief source of financial support for "small science" in British universities, says that, in future, industrial applicability will be one of the chief criteria for deciding how research grants will be awarded, thus loading the dice against academic scientists whose interests lie elsewhere. When fashion and prejudiced opinion have such an important influence on the academic market, it would not merely be unfair to expect that academics should allow their careers to be determined so arbitrarily, but would probably be impossible: potential academics would prefer to look for jobs elsewhere.

Implications

None of this implies that the tenure system as it is is sacrosanct. On the contrary, there is both a need for change and evidence that universities and those who work in them agree. Plainly, if a university as such should cease to exist, it would be unreasonable of its employees to expect that their employment should nevertheless continue. Similarly, if a university should find that student demand for some of the services it offers has collapsed, and those concerned are unable to discover other opportunities to exploit, it is only seemly that they should be invited to pursue their academic interests in some other environment. For while universities are, or should be, self-governing communities of scholars whose unique product is the outcome of successful research, their bread and butter, now as in the twelfth century, is the education of students. But the revision of the rules of tenure which the British Government is seeking will be equitable and tolerable only if it is accompanied by a restoration of some semblance of each university's freedom to decide for itself what to teach, and to whom.

The specific implications of this simple rule are plain. First, the student quotas, which are unfair to would-be students and a source of serious diseconomy, should be abolished. Since the system exists only because the government cannot think of any other way of limiting the aggregate amount of what it is required by law to pay to local authorities in respect of university fees and maintenance grants, it should quickly bend its ingenuity to helping young people now denied higher education to pay for it. Second, the market in higher education should be made to work freely in the sense that universities should be rewarded according to their effectiveness in satisfying the demands made on them. As things are, individual universities may find departments of which they are proud snatched away because some committee has decided that another arrangement would better meet the national interest. Finally, for restriction of tenure to be equitable and acceptable, universities must not merely be free to make academic decisions for themselves (with the risk of being wrong some of the time), but must do so in such a way that those whose careers are affected fully participate.

If these conditions could be met, the heavy-handed mechanism the British Government now plans to use for the enforced restriction of tenure (on new appointments only) would not be necessary. Moreover, some of the devices that have been suggested for amending the standard form of academic tenure — longer probationary periods or even periodic reviews — would be seen to be irrelevant, even counter-productive. For younger academics, and those whose chief contributions to the work of their universities is in teaching rather than research, are just as much in need of protection from tyrannical superiors as those whose research is off the beaten track. Thus the standard terms of tenure should be amended only so as to allow appointments to be terminated when a university is forced by financial considerations to contract or when, after serious and open discussion within an institution, a change of academic policy seems prudent. The need to demonstrate due process should be paramount.

Even at this late stage, the British Government should recognize that it is more likely to damage its university system beyond repair by forcing amendment of academic tenure through the parliamentary system than to gain from winning a symbolic fight with institutions already all too painfully aware of being in its pocket. Victory, of course, would allow the government to claim that it had put academics on the same basis as other professional people. Only with the passage of time would it become apparent that the supply of would-be academics had dried up. Indeed, it is even possible that signs of damage would never become so obvious that the government would be compelled to notice. Good people, notoriously mobile as things are, would seek posts in research institutes or universities elsewhere, allowing vacancies to be filled by the less able. Is that what the British Government wants? □

Banking under strain

The near-collapse of a US bank shows the need for a deal with developing countries.

THE unwelcome shadow of the Great Crash of the 1930s was abroad like the reaper last week, and it is too soon to know whether it has gone for the time being. Just as the First World War began with an assassination in the obscure Serbian city of Sarajevo, so the decade of economic upheaval of the 1930s began with the collapse of an obscure Austrian bank of which most of those affected had not previously heard. Last week's difficulties were inevitably better publicized. On the Monday, a group of New York banks announced that they had clubbed together to provide $4,500 million of credit to the Continental Illinois Bank and Trust Company, a kind of bankers' bank which has so far made its way in the world by borrowing on a short-term basis from other banks and lending on a long-term basis to other kinds of institutions, mostly commercial companies. After two disastrous years, partly attributable to a rash of light-hearted lending to oil-exploration companies (through the intermediary of an Oklahoma bank which collapsed in July 1982), the bank's bad debts had grown to more than $2,000 million, more than the value of its capital. So, inevitably, other banks became chary of lending to Continental Illinois, which is said to have been borrowing overnight money from its fellow banks to the tune of $8,000 million each day. By this yardstick, it is hardly surprising that even the $4,500 million was not enough to still the rumour mills, so that by the end of the week the US Federal Reserve, the lender of last resort, was forced to chip in a further $7,500 million to prevent Continental Illinois from going bust.

Why should that matter? Why should the Federal Reserve have gone to such trouble to save an over-ambitious bank from collapse when there are many in Congress who think it high time that the banking industry, not noticeably unprosperous, was taught a lesson? And why, if it comes to that, had not the Federal Reserve diligently exercised its supervisory powers to ensure that Continental Illinois lived less hazardously? All the answers are implied by a ringing declaration put out last weekend by the principal debtor nations of Latin America — Argentina, Brazil, Colombia and Mexico, which between them have external debts of $240,000 million. In the past two years, each of these governments has been compelled to ask its creditors for more time to pay, and for extra help (from the International Monetary Fund), and as a consequence has had to accept deflationary domestic policies. (In Argentina, for which a rescue package has still to be arranged, the rate of inflation is now 480 per cent year.) At the weekend, the four governments declared that they "cannot indefinitely accept" that "the aspirations of our peoples for development, the progress of democratic trends in the region and the economic security of our continent" should be put in hazard by high interest rates on borrowed funds and by the protectionism now apparent to suppliers of primary raw materials. This telling statement explains what has been happening in the United States because it draws attention not merely to the plight of the debtors but to the vulnerability of their creditors. The commercial banks could fall like dominoes if, in these delicate circumstances, one of them keeled over, while banking's supervisory agencies, accustomed as they have become to letting banks count as assets even their most dubious loans to developing countries in money trouble, plainly cannot be over-diligent in regulating the banks' domestic business.

The reasons why this underlying delicacy has so suddenly become apparent are also simple, and are to be found in Washington and New York. The administration's huge budget deficit, in round numbers £200,000 million this calendar year, has somehow to be matched by persuading people somewhere to buy US Government securities. If the United States were financially isolated, the result would be a high rate of domestic inflation. In the real world, much of the borrowed money comes from elsewhere, from overseas investors attracted by the high rate of interest the federal government must pay and also by the signs there have been, in the past year, of a return to rapid economic growth. The result of all this is that the dollar has been strong relative to other currencies and that the terms of trade have moved against the United States, which is likely this year to spend $80,000 million more on imports than it will earn from exports (which will again be financed by borrowing from abroad). But an election year is hardly the time to expect the federal government to change its tack.

Where all this will lead is anybody's guess. To the extent that Continental Illinois will now have been able to replace some of its short-term debt, interest rates should fall temporarily but at the expense of the inflation rate (which will be slower to respond). Sooner or later, there will have to be a settlement with Continental Illinois, in the course of which the bank's shareholders will be the chief losers (the consequences of which will be modestly deflationary). Whether these mildly beneficial effects will last until the presidential election in November is uncertain; most probably, they will not, for the commercial banking system will long before then be grappling with the next crisis among the debtor nations. To judge from the weekend declaration, that negotiation will be more tricky than the successful attempts to reschedule the payments of debt which there have been in the past two years, when the commercial banks have safeguarded their assets (loans) by lending debtors part of the money required to pay past interest, relying on tough economic recipes dictated by the International Monetary Fund to help ensure that the amounts outstanding will also in due course be paid.

What the Latin-American debtors now say is that they cannot continue to toe the bankers' line. Instead, they plead that there should be a conference later in the year to consider ways in which they could earn their way out of trouble by being helped to sell raw materials to their industrialized creditors. The catch is that the volume of debt is far too great to make that a practical proposition, even though, on this occasion, the commercial banks will be eager for any help that such a device could bring. The stratagem of replacing all the debtor nations' obligations by long-term loans from some international organization, which would safeguard the commercial banking system, is in many ways objectionable, but may be the only course open to the industrialized West when it holds its summit meeting in London next month. □

Direct broadcasting satellites

Japanese fury over US component failure

Tokyo

THE failure of US-assembled components in Japan's Yuri 2A satellite — the world's first direct television broadcasting satellite (DBS) — has provoked a furious reaction from the Japanese Government. In language normally reserved for serious clashes over trade imbalances, the Minister of Posts and Telecommunications, Keiwa Okuda, told visiting US Vice-President George Bush last week that the government took a "serious view" of the breakdown of two out of three of the satellite's transponders.

Yuri 2A was launched in January to improve reception of the state broadcasting system (NHK)'s two television channels for around one million people living in mountainous regions, remote islands and high-rise "urban canyons". But with only one transponder functioning, NHK has abandoned plans to broadcast its second educational channel. A spokesman for Toshiba, the satellite's main contractor, said the malfunctioning units were manufactured by Thomson CSF of France and assembled by General Electric in the United States.

The angry reaction to the failure does not, however, reflect disappointment at the loss of educational broadcasts but the critical role Yuri plays in a whole set of conflicting aspirations for the Japanese telecommunications, satellite and electronics industries.

All the technologically-advanced nations are now making plans to introduce direct satellite broadcasting. By being first off the mark, Japanese manufacturers had hoped to establish themselves as the number one suppliers of receiving equipment. The parabolic antennas and decoders needed to receive NHK broadcasts are already on sale in every corner electronics store in Japan, and Toshiba and a Nippon Electric Company (NEC) affiliate have been named as suppliers by Satellite Television of the United States, which hopes to have its own satellites ready by the end of 1985. The failure of Yuri is bound to affect sales in the home market, which would have built up experience for the international market.

The same companies are also eyeing the much bigger technological opportunities to be offered by DBS in the future. Japanese companies believe that DBS will be used in the 1990s to begin high-definition television broadcasts. Already Japan is the world leader in this technology. As long ago as 1968, NHK research laboratories had produced a system with 1,125 lines — almost twice the number currently in use in the United States and Japan (525) and in Europe (625) — which gives a picture no different in quality from commercial 35-mm movie projection. The trouble is that this NHK technological advance can only be capitalized upon if the 1,125-line system is adopted as an international standard. Strong competition is now coming from a 925-line system developed in the US Advanced Television Project at Massachusetts Institute of Technology. Technically the US system looks superior because it requires less transmission capacity.

Both Sony and Matsushita Electric will have 1,125-line systems ready for the marketplace by 1986. And by getting working systems out as soon as possible — beginning with mini "video theatres" — they hope to push international acceptance of the 1,125 standard. Any slowing in the pace of satellite development is seen as a long-term threat to the technological lead they will have when DBS becomes available for high-definition television.

More immediately worried by Yuri's failure are big businesses which want to buy communications satellites to transmit their own business data. Only a year ago, Keidanren (the Federation of Economic Organizations), backed by the satellite building lobby, was insisting that all Japan's satellites be domestically developed and the goverment was refusing to permit businesses to import satellites. It was a combination of pressure from the United States, which saw yet another import barrier, and from businesses which want to use satellites and know that it will be far cheaper to buy them ready-made from the United States, which forced the government to change its mind at the end of last year and to allow at least communications satellites — if not other categories — to be imported.

The failure of Yuri now has the domestic satellite makers screaming for foreign imports to be stopped again — on the grounds that they are too unreliable. But the potential users have already formed a 30-company group to purchase a communications satellite from the United States. Their counter-argument is for a domestic satellite which will still have to contain a high percentage of foreign parts.

Criticism is also coming NHK's way for having the satellite built with exceptionally powerful transponders. This move had been strongly opposed by engineers favouring the use of already-tested designs but had gone ahead to make it possible for the transmissions to be received on smaller antennas. Now NHK is wondering what to do with Yuri 2B, an identical satellite scheduled for launch in the summer of next year. Should it be rebuilt or launched as it is?

For the time being anyway, NHK is cutting its losses by licensing the satellite as an "experimental" rather than "broadcasting" service with the Ministry of Posts and Telecommunications. No charges will be made for those who have bought antennas and are receiving Yuri's fitful broadcasts.

Alun Anderson

Copyright for software in Australia

Canberra

THE Australian Government has announced its immediate intention to extend to computer software the copyright protection usually given to literary works. This should end the present uncertainty about the legal status of software following the decision of Mr Justice Beaumont in the APPLE COMPUTER *versus* COMPUTER EDGE case late last year that certain types of computer software were not protected by copyright under the 1968 act. The case is still under appeal before the full federal court.



A joint statement issued on 15 May by the Attorney-General and by the Ministers for Industry and Commerce and for Science and Technology, says that the intended inclusion of software in the existing copyright category of "literary works" rather than under the less restrictive patent law is intended to stimulate research and development in the local software industry.

Unless a federal full court decision supervenes, it is intended that passage of the proposed legislation should be secured in June, and should be understood as a short-term measure. It will not pre-empt consideration of fundamental issues remaining to be resolved, both at the domestic and international levels, on long-term policies for the protection of software and works stored in or created with the aid of computers.

Jeffrey Sellar

Genetic engineering

Rifkin wins interim injunction

Washington

AN experiment that would have been the first to release recombinant organisms to the environment was blocked last week by a federal judge (see p. 301). Granting a motion filed by anti-genetic engineering activist Jeremy Rifkin, Judge John Sirica ordered the University of California not to proceed with the experiment of Drs Steven Lindow and Nikolas Panopoulos until a full hearing can be held on Rifkin's lawsuit, which challenges last year's approval of the experiment by the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health (NIH). RAC was also enjoined from considering other proposals for deliberate release of recombinant organisms while the case is pending.

The Lindow experiment was to have tested the ability of altered strains of the bacterium *Pseudomonas syringae* to protect potato plants in the field against frost damage. The altered bacteria have a deletion for the gene responsible for an ice-nucleating protein excreted by the wild bacteria on the leaves of plants.

Attorneys for the University of California said they would immediately appeal. According to Dr Lindow, if the experiments cannot begin this week or next, they will be delayed until the autumn, when temperatures at the test site in northern California once again drop below freezing. The experiments, originally planned for last autumn, were postponed when Rifkin threatened at that time to seek an injunction (see *Nature* **306**, 349; 1983).

Sirica's unusually detailed ruling on the preliminary injunction strongly suggests that he will also find in Rifkin's favour when the hearing is held. Rifkin is claiming that NIH should have filed environmental impact statements before approving the Lindow experiment. The ruling does not affect private industry, but only experiments carried out with NIH funds. Advanced Genetic Systems has submitted a proposal to RAC for an experiment virtually identical with Lindow's which is likely to be approved at the next meeting of the committee.

Although the ruling is a victory for Rifkin, Sirica made clear that he was making no judgement on the scientific issues that Rifkin had tried to raise. Sirica's judgement says that he was deciding only "narrow legal questions" and, during last week's hearing, he repeatedly rebuffed attempts by Rifkin's attorney to present testimony from scientific experts.

If Sirica finds for Rifkin in the full hearing, the immediate effect will merely be to require an environmental impact statement, but Dr William Gartland, executive director of RAC, said that process would take a year or more. The consequences might be that investigators would meanwhile carry out their experiments in other countries — or industry-sponsored research would proliferate.

Lindow's experiment, first considered in October 1982, was unanimously approved at the April 1983 meeting of RAC. During its brief deliberation, the committee noted that Lindow had already performed identical experiments involving chemically-mutated bacteria without ill effect and that the experimental proposal had been modified to meet earlier concern.

On both occasions, the proposals were published in the *Federal Register* for public comment; none was received on either occasion. According to Sirica's ruling, NIH made two mistakes in handling the matter. First, they failed to prepare a "programmatic" environmental impact statement in 1978, when the guidelines were modified to permit deliberate-release experiments at the NIH director's discretion.

Second, they failed to carry out even an "environmental assessment" of the Lindow experiment. Sirica rejected the US Government's argument that RAC's deliberations constitute an environmental assessment, saying he was "confident that the plaintiffs have identified several areas of plausible environmental concern which are not rebutted on the record before this Court".

Stephen Budiansky

Andrei Sakharov

Birthday gloom and celebrations

New York

LAST Monday, 21 May, the 63rd birthday of Dr Andrei Sakharov, the dissident Soviet physicist now in internal exile in Gor'kii, was marked by various and diverse events. In the Soviet Union, Dr Sakharov himself, who with his wife has been on an open-ended fast since 2 May, was removed, over the weekend, to a nursing home, where he is reportedly being forcibly fed. At the same time, the Soviet media stepped up their attacks on Sakharov's wife, Elena Bonner, on whose behalf he has undertaken the fast, accusing her, in effect, of exploiting her husband. In the United States, however, the day was marked by a concert in New York's Carnegie Hall, and the conferring *in absentia* of two honorary degrees, by the Universities of Long Island and of Pennsylvania.

The concert and the degree ceremonies were, of course, planned long ago. Nor is this the first time that Sakharov has received an honorary degree. Moreover, some of the formal speeches placed relatively little emphasis on the current plight of Sakharov and his wife, but delivered very much the same message on the scientist's responsibility to society as they would otherwise have done.

Sakharov's physicist colleagues, however have responded to the latest events. Last week, 36 members of the US National Academy of Sciences (of which Sakharov is an honorary foreign member), including 13 Nobel laureates and the presidents of the American Academy of Arts and Sciences and the American Physical Society, sent a cable to the Soviet leader, Konstantin Chernenko, stressing their "deep concern" and saying that the death of either Sakharov or his wife would create "very severe and long-lasting damage to efforts to maintain and to improve collaboration and cooperation with our Soviet colleagues". A "humanitarian act to save the Sakharovs", rather than "the creation of martyrs" is, they urge, "surely the common concern of all".

The Soviet view, however, is that they are *not* creating martyrs, but forestalling an anti-Soviet plot. Adequate treatment for Mrs Bonner's heart condition is, they say, available in the Soviet Union. Her demand to go abroad (the repeated refusals of which triggered the fast) is, according to TASS, the official Soviet news agency, only so that she might become "one of the leaders of the anti-Soviet scum on the payroll of the Western special services".

As early as 4 May, TASS described a scenario in which "with the participation of American diplomats. . . Sakharov would declare another 'hunger strike' while Bonner would receive 'asylum' in the US Embassy in Moscow" for the purpose of meeting foreign correspondents and "for the transmission abroad of slanderous fabrications about the Soviet Union. This "operation", TASS claimed, was "frustrated" by the "timely measures taken by the Soviet law-enforcement agencies".

The US Embassy in Moscow and the State Department in Washington immediately denied any US involvement in such a plan. Last Saturday, however, the *New York Times* quoted unnamed State Department officials as admitting that Sakharov had appealed to the embassy to shelter his wife during the fast but that, since the embassy had not received Sakharov's letter until after Mrs Bonner had left Moscow for Gor'kii, they had not discussed either the appeal or the fast with her. The same issue of the *New York Times* also carried a story, originating from Jean Daniel, editor of the Paris weekly *Le Nouvel Observateur*, claiming that there had been "indirect" approaches from Soviet diplomats suggesting that the Sakharovs would be allowed to leave the Soviet Union if President François Mitterrand of France called for a halt to the deployment of medium-range nuclear missiles in Western Europe by the North Atlantic Treaty Organization (NATO).

Vera Rich

UK research support

Industrial promise to be blessed

THE science board of Britain's Science and Engineering Research Council (SERC) has given notice to the research community of how it wants to develop its new "active" research funding policy. "Clear industrial potential" seems to be one guiding principle. The board has drawn up a novel classification of the research it finances and has initiated reviews which, it claims, are already suggesting neglected areas.

In a policy statement published last week, *A strategy for support of core science*, the board says that reviews being carried out by its separate committees stress the importance of access to advanced instrumentation and adequate provision of personnel. In chemistry, where the most progress has so far been made, the synthesis of new materials and the study of catalytic reactions at surfaces will receive a higher priority, while low-dimensional structures, protein engineering and chemical sensors are among interdisciplinary areas identified as "research themes" deserving more support. For biology, image-processing and neuroscience are short-listed priority areas.

The science board is the largest of the four main arms of SERC and finances research worth £70 million a year. But it has been under increasing financial pressure in recent years, and this has emphasized the need to take a more directive role in selecting research areas, according to its chairman, Professor John Cadogan. Research grants to university workers, for example, fell from £24 million in 1977–78 to £19.5 million in 1982–83 (1982 prices), and 30 per cent of grant applications deemed worthy of "alpha" status are now going unsupported. The remainder of the science board's budget divides roughly equally between supporting research students and maintaining and developing central and regional facilities such as the synchrotron radiation source at Daresbury, Cheshire, and the central laser facility at the Rutherford-Appleton laboratory in Oxfordshire, as well as university installations. Cadogan is quite candid about his aim of securing a bigger slice of SERC's budget for the science board.

Many feel that astronomy and nuclear physics, each of which has its own board within the research council, are overrepresented on SERC's governing body. A recent proposal to merge them was dropped despite the personal backing of SERC's chairman, Professor John Kingman. Cadogan, an industrial chemist, is diplomatically noncommittal, suggesting that maybe the time was not right. But the failure of the idea will not have made his crusade for core science any easier.

The new strategy is an attempt to get away from the system used to assess grant applications before 1982, when each was assessed strictly on its own merits. Now the board will decide what the priority subjects should be, although it stresses that there will be continuing reviews.

It seems possible that in future more research with clear economic potential will be supported. Here the new strategy document appears somewhat uncertain. "It is not the science board's job or intent to support research specifically for industry", it declares boldly, adding, however, immediately afterwards that it "believes it must support core science in such a way that British industry prospers". Nevertheless, the board insists, it remains a fundamental tenet of its philosophy that funds must be made available for "outstanding scientists" on any problem of "excellence, timeliness and promise". The idea of a special fund for such "blue sky" research was dropped, however.

The science board's most urgent priority now is to increase again the amount that it can spend on research grants, and a strong case along these lines is contained in SERC's submission to the Advisory Board for the Research Councils on scientific opportunities for 1985. Something of the new subject strategy exercise has also found its way into this document, although the full impact will not be felt until next year's survey of opportunities for 1986. In the meantime, the details of "research themes" in most subjects are still being worked out, and the science board hopes its new document will be circulated widely and attract comment. **Tim Beardsley**

Education in Canada

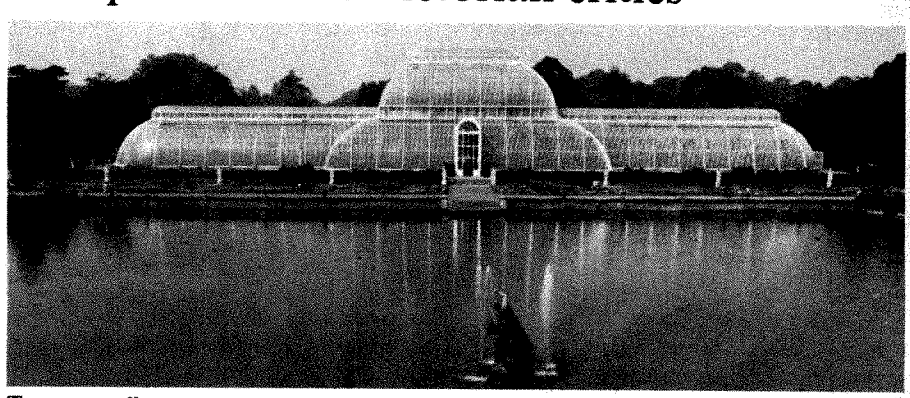
Washington

THE Science Council of Canada has joined the international stampede to declare the need for "sweeping" changes in the ways its schools teach science. In a new report for the Ministry of Science and Technology, the council says that most teachers are underqualified and that science textbooks and curricula present a picture of science so oversimplified that it is no longer authentic. Unlike a similar report published last year by the United States' National Science Board, however, the Canadian study claims that dramatic improvements can be made without large expenditure and by working through existing institutions.

The report's principal recommendation is that all elementary school children should receive science education as proposed in the official policy statements of provincial ministries of education. The Science Council concluded that these policies are seldom observed and that most children are given only "token" exposure. The council is impatient with the argument of many elementary schools that science is "integrated" in other class activities, believing "integration" to be so vague as to be of little value. It says science should be taught as a specific subject for at least 10 per cent of the time.

Another major issue raised in the Canadian report is the quality of science textbooks. The council does not quarrel with their accuracy, but says they fail to convey a realistic sense of the way in which research is conducted and of the link between science and technology. Peter David

Kew palms off its Victorian critics



THE normally serene surroundings of the Royal Botanic Gardens at Kew, on the outskirts of London, have been rudely disturbed in recent months.

Bitter arguments have raged over plans largely to reconstruct the outstanding 19th-century palm house (above), designed by Richard Turner and Decimus Burton and the inspiration for tropical plant houses the world over.

The building is in poor structural condition and now has to be painted every two years, while 600 panes of specially curved glass have to be replaced annually. But renovation plans put forward by the Property Services Agency of the Department of the Environment have aroused the ire of the Victorian Society, among others. The existing wrought-iron glazing bars will have to be replaced with stainless steel, because wrought iron is not now available in sufficient quantity.

Some sort of compromise on the scheme has now been reached. But the Garden History Society is still unhappy with plans to increase the extent of the plant beds and so cover the iron grating floor, although the plants grow best in beds. Tim Beardsley

Silicon manufacture

Monsanto sets up in Britain

THE United States chemicals group Monsanto last week announced plans for a £65 million investment in a new British silicon wafer research and production operation. The intention is further to improve crystal purity, which will involve developing new instrumentation, and eventually to manufacture wafers of up to 8 inch (200 mm) diameter. Monsanto expects to collaborate extensively with British university departments. World supplies of silicon wafers manufactured to the required purity and high degree of flatness are at present restricted to relatively few manufacturers. Demand is expected to increase rapidly, however. Monsanto is a leading supplier to the semiconductor industry in the United States but lags in Europe. The new Monsanto facility will be at Milton Keynes, a new development town in central England. The company explains that it was looking for a green-field site, which Milton Keynes possesses in abundance, and that Britain was chosen from a short-list of eight European countries. The British Government has helped with grant assistance, although the amount is not being made public.

In declaring its intention to move towards 200-mm wafers, Monsanto seems to be making the point that it wants to keep two steps ahead of the competition: elsewhere, 150 mm is proving difficult. Monsanto boasts leadership in producing 125-mm wafers in a peak-to-valley flatness of less than 2 micrometres. But as modern wafer-stepping semiconductor manufacturing techniques become more widespread, edge-to-edge flatness will cease to be a major constraint. Local slope and crystal purity may become the limiting factors to cramming semiconductor devices onto wafers.

Two trends are combining to make purity a problem. First, chips are becoming larger, so that there is a decreased chance that each chip will be flawless. Second, channel widths are now moving down to the order of micrometres so even very small local deformities in crystal structure can disrupt a device. Implanted ions tend to spread from their original deposition sites during the manufacturing process, for which allowance has to be made in the design. The extent of the spread is determined by the structure and purity of the original lightly-doped crystal, and this is where the thrust of the research effort is likely to be. Initially, Monsanto will import wafers from the United States for cleaning and polishing in Britain, but will later embark on crystal manufacture, hoping eventually to supply 50 per cent of the British silicon market. **Tim Beardsley**

Research councils defend manning

SIR Hermann Bondi, chairman of the Natural Environment Research Council (NERC), appeared before the Public Accounts Committee of the House of Commons on 14 May to give a doughty defence of his council's manpower planning. The National Audit Office recently criticized research councils and the Department of Education and Science for apparent laxity in their planning controls (see *Nature* 309, 104; 1984), and suggested that there had been an unexplained upward drift of salary grades at councils including NERC. But, according to Sir Hermann, the increase in the number of senior posts was a planned development and a completely appropriate outcome of the changing role of NERC.

Sir Hermann said that over the past 10–15 years the need for lower-grade NERC staff on foreign aid programmes had decreased, as local inhabitants had been trained to take over their jobs. The simple result was that the staff NERC retained were now in higher grades. He also asserted that NERC institutes such as the Institute for Marine Environmental Research were deliberately established with young scientists who would climb up the ladder of promotion.

Sir Hermann also pointed out that scientific equipment had become more complicated, so that many measurements that were formerly made manually are now made by computer. This also led to a smaller requirement for lower-grade support staff.

He went on in stirring style to turn the tables on MPs who had been led to doubt whether the research councils were discharging their manpower planning duties effectively. The main charge of the National Audit Office was that the research councils had not been keeping their staff grades in line with those in the civil service. The Public Accounts Committee was particularly worried about "fluid grading" of science research posts. Sir Hermann's ingenious reply was that manpower planning in NERC is better than that in the civil service. The in-house system of visiting groups, promotion boards and annual reviews was all that could be desired.

The committee's next witness, Dr Ralph Riley, secretary of the Agricultural and Food Research Council, said that the controls on manpower in his council were similar to those at NERC, so much the same arguments applied.

The committee appeared well pleased with these answers. But research councils have not escaped completely unscathed. Mr David Hancock, permanent secretary at the Department of Education and Science, used the occasion to announce a "thorough review" of fluid grading. Tim Beardsley

US high-technology investment

Partnerships lose shine

Washington

THE downturn in high-technology stock prices is hampering efforts by biotechnology companies to raise research and development funds through other routes as well. Last week, Biogen revealed that it has raised only $25 million of the $40 million it had been counting on for a research and development partnership to finance the company's commercial development of gamma-interferon and interleukin-2. In recent weeks, other biotechnology research and development partnerships have met with a similar reception from investors. Hybritech was able to raise only half of the $80 million it was aiming for; and Centecor, which had hoped to raise $10–$15 million, now says it will be happy with $5 million.

Research and development partnerships are attractive to companies because they can provide stable support for long-term research and because they are a source of capital which does not dilute the equity holding of existing shareholders. Private investors obtain immediate tax benefits and later receive a share of the royalties from any inventions arising from the research. The partnerships are especially useful to emerging industries such as biotechnology which do not yet have products on the market and thus do not have a reliable source of revenue. They also give the companies far greater freedom than they would have if they entered into agreements with a single corporate research sponsor.

In the Biogen offering, the company ended up putting $35 million of its own money into the research and development partnership, considerably more than the $20 million it had planned.

The lack of enthusiasm for the partnerships reflects several developments. Biotechnology stocks have not been doing well on the market lately. What is more, several limited research and development partnerships in the computer field have recently received unfavourable attention for their failure to produce commercial products, which has tended to give all high-technology ventures a bad name. The most prominent case was that of Storage Technology, which raised close to $100 million for a research and development partnership that was to produce a mainframe computer to compete with that of IBM.

Nelson Schneider of E.F. Hutton says that another factor behind the poor reception for research and development partnerships in biotechnology is that there is now much more competition for the investors' dollars. "There are literally dozens out there in the market in the $2–10 million range in biotechnology", he says. **Stephen Budiansky**

Australasian science

Going all the way with B.J.

Canberra

THE 54th Congress of ANZAAS, the Australian and New Zealand Association for the Advancement of Science, was held amid the fading autumn colours and falling leaves of the spaced-out campus of the Australian National University. For five days from 14 May, the congress generated wide coverage of popular items in the press and on radio. Yet the congress lacked the focus promised by the stated theme of "Horizons of Science". The congress was woolly at the edges and soft at the centre.

For decades, the peripatetic congress has struggled to resolve the inherent tension of providing both parallel specialist meetings and cross-disciplinary exchange. Other conflicts surfaced again, notably the confusion about objectives. Is ANZAAS primarily seeking to communicate the latest results, ideas and challenges in science to the wider public and, incidentally, to persuade politicians of the importance and relevance of science? Or is the event principally for academic discourse? At Canberra, neither promise was fulfilled.

Among the 1,600 delegates — a big drop from earlier meetings but still the largest meeting on the Australian academic calendar — were relatively few who had come to listen and to discuss. With more than 1,000 papers being delivered, the speakers outnumbered others. People from outside the academic or research spheres were notably absent. To reach the public at large, or even groups such as schoolteachers and students, ANZAAS relies entirely on journalists.

The trend away from science and technology towards the social sciences and humanities continued. Of the 33 specialized sections, only 15 were devoted to the sciences and technologies, and some were pitifully weak.

The "Horizons of Science" theme, only dimly reflected in the congress programme, was almost totally ignored by individual speakers. Thus a showcase for the current state of Australian science was left empty. But some of the congress-wide symposia produced fruitful discourse, tackling issues of current concern in Australia which as exotic diseases, science and political decisions, technology strategy and nuclear arms. The last, with Petra Kelly and retired General Gert Bastian, parliamentary members of the West German Green Party as visiting attractions, was the best attended, reflecting the growth of the peace movement in Australia.

This symposium even produced a collective statement by a working group on the effects of the British nuclear tests carried out in the Australian desert between 1952 and 1963. Allegations of a cover-up by the British of aspects of these tests have become the centrepiece of a major diplomatic row between the Australian and British Governments and the rallying point for anti-nuclear protests in Australia. The ANZAAS group called on the Australian Government to conduct a comprehensive interdisciplinary study of the effects of the British tests, saying that a committee formed by the Department of Resources and Energy is inadequate.

ANZAAS was not entirely at odds with the federal government, however. Professor Sir Gustav ("Gus") Nossal, this year's president, gave one of the most glowing tributes to a politician by a scientist in recent times. An immunologist and director of the Walter and Eliza Hall Institute for Medical Research in Melbourne, he solidly backed the discussion draft released in April by Mr Barry Jones, Minister for Science and Technology (see *Nature* 3 May, p.6).

The draft, he said, was "the most significant and courageous document to come out of Canberra on any topic in the past decade". The ANZAAS president went on to urge that the minimum realistic incentive for industry to increase its commitment to research and development would be a tax credit of 150 per cent.

Sir Gustav saw "the most significant, marvellous and stunning element" in the technology strategy draft as being the proposals for achieving 50 per cent completion of secondary school (from 36 per cent now) and 20 per cent of tertiary education (a doubling of the present figure) by 1995. While critical of the emphasis on the quantity of education and of the view that the draft was "singularly silent on quality", Nossal hs put his reputation on the line as an influential backer of Jones.

Not everybody in Canberra approved. Professor Gordon Reid, until recently at the University of Western Australia and soon to be governor of the state, said Sir Gustav's statement was not based on scientific inquiry and accused him of "exercising hyperbole in his role as president".

Professor Reid said Sir Gustav's statement was an example of the ambivalence of Australian science. In one breath, he said, scientists plead for freedom from government direction, and in the next for more financial support.

Nossal is unrepentant. "A tremendous challenge is faced by the Australian scientific community to explain ourselves better. . . and to promote a technological culture. We scientists should inform the public about what we are doing, about what practical results can realistically be expected, and about what longer-term dreams can legitimately be espoused."

Later, he complained of "the besetting sins of Australian scientific academics of treating PhD students as clones of themselves and of shunning applied orientations for their research". He asked university administrators to treat their relations with the media and the public "seriously and as a sacred duty".

By defining its role in terms of facilitating communications among scientists and between scientists and the public, ANZAAS might be expected to take a lead in solving the problems posed by its current president. In reality, ANZAAS has little to offer between congresses as its central organization is weak and strapped for cash (it receives no government support and has fewer than 3,000 subscribing members).

However, bold plans for change were announced by the organiizers of the next congress, to be held in Melbourne in August 1985. They will virtually abandon the specialized sections and, through a broad grouping of interests under seven headings, will redirect their offerings to young people and the general public (both of which groups were noticeably absent from the Canberra congress) while attracting scientists to interdisciplinary sessions which will explore the current "state of the art" and identify promising new directions.

Peter Pockley

Nature banned (1937)

As part of the continuing availability of German documents of the 1930s, it has come to light that the National Socialist government issued a decree, circulated to all university and research libraries, requiring that copies of *Nature* should not be made generally available. Moreover, the decree required that its own existence should be secret.

The text of the decree, dated 12 November 1937, is as follows:

> **Articles are often published in the London weekly scientific journal *Nature* containing outrageous and vile attacks on German science and the national socialist state. The journal must therefore be excluded from general use in scientific libraries. I request the university, academies, institutes and seminar libraries to carry out the appropriate steps. Regarding the sequestration and restricted use of the journal I refer to my decrees of 17 September 1934, 3 April 1935 and 16 December 1936, UI 22733, W Ie 828 and W Ia 2305.**

The decree is signed by the Imperial and Prussian Minister for Science, Education and Development and no doubt refers to the continuous stream of hostile comment appearing in *Nature* in the mid-1930s, much of it centring around the work of the Academic Assistance Council formed in 1933 to help German academics to find posts overseas. Thus in July 1937, *Nature* (140, 169; 1937) referred to the dismissal of German Jewish academics as the "German disaster" and remarked that it was unlikely that "the leaders of intolerance and irrationalism will be deterred by the knowledge that their victims will be sheltered in the asylum of liberal countries". □

Indian science circus

SIR — While your comprehensive reports on science in India in the issue of 12 April are true and appropriate, I should like to stress some of the basic problems behind the prevailing confusion afflicting the Indian science scene.

Modern scientific education and research struck roots in India during the early twentieth century and rapidly grew after independence. The first scientists who were patronized by the government had their own individualistic ideas on developing science in India, for which most of the equipment had to be imported. Soon after independence, India suffered a shortage of foreign exchange, which hindered the import of adequate support materials, but the number of scientific institutions nevertheless continued to increase. This odd situation led to a lowering of the standards of scientific activity. However, many students could imbibe bookish knowledge and some of them could easily migrate to other countries where opportunities for work were abundant. Thus, Indian scientists are thriving both at home and abroad, but occasional soul-searching goes on and comparisons between home-based and expatriate Indians are sometimes not well chosen.

It requires a great deal of patience and faith to work in Indian conditions, in which even essential items are perpetually scarce. Time and efficiency are of relatively little importance in our country and hero worship, blind or forced, is part of life. Unfortunately, modern technologies need less hero worship and more efficient practice. However, our policy-makers (heroes) are not deterred from buying or borrowing any latest technology, and camp-followers are easily found among our vast multitudes. Thus, India constitutes a country of tremendous contrasts wherein basic facilities such as power supply, telephone and other communications services are woefully inadequate on the one hand, while on the other, huge installations for research in space and biotechnology are being planned without the least hesitation. Just as the highly rich and the abjectly poor exist side by side, so also do high and low technologies. After all, ancient emperors built great monuments like the Taj Mahal, probably employing a starving labour force, and as if to keep up that tradition, our science administrators in the past few decades have also been able to build a few glamorous edifices in the name of excellence.

Our tolerance is a boon, giving us the willpower to live amidst glaring disparities, but it is a bane to the implementation of contemporary developments. Finally, it is another wonder of the world to watch the mystic religious practices that cost next to nothing and modern scientific pursuits at exorbitant costs blissfully coexisting in India!

C. RADHAKRISHNAMURTY
Tata Institute of Fundamental Research, Bombay 400005, India

No thanks to. . .

SIR — So you think that the Royal Society "has broken new and dangerous ground" in the acknowledgements by authors of papers published in the society's journals (*Nature* 26 April, p.762). What is so new? And why so dangerous?

The practice of acknowledging help from others goes back a long way, even in this society's history, so it is certainly not new. If authors are now a little more generous and a little less formal than in the past, why is that so dangerous? The law of libel should remind editors not to allow to be printed the sort of remarks that you fear could be dangerous.

A paper in *Phil. Trans.* (1883) concludes "My best thanks are due to Professor Lankester for some excellent specimens of *Paragorgia, Villogorgia* and *Briareus*, and I am also deeply indebted to Professor Mosely who freely placed his numerous preparations at my disposal and whose constant aid and advice have been of invaluable assistance to me." The acknowledgements from *Proc. R. Soc.* (1984) that you quote are in a long and honourable tradition.

R.W.J. KEAY
Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK

● Lankester's organisms are Alcanarians (sea anemones). The more recent acknowledgement that seemed to break new ground read "This research was not supported by any military organization". — Editor, *Nature* □

Publishing chronology

SIR — We read with interest the piece by John Maddox under the title "Extinctions by catastrophe?" (*Nature* 19 April, p.685). The complex of problems produced by the distribution of preprints and by prepublication press coverage is certainly something we should all ponder. It is clear that the system is sometimes beneficial and sometimes not and that there are no simple answers.

In condensing a complex chronology, however, one non-trivial item was left out. In August of 1983, we presented a summary of our research on periodic extinction at a widely attended meeting on the "Dynamics of Extinction", held in Flagstaff, Arizona. The oral presentation was accompanied by a printed abstract as part of the official programme for the Flagstaff meeting. The meeting was attended by several science writers and subsequently *Science* (**221**, 935; 1983), *Science News* (**124**, 212; 1983) and several other publications carried accounts of the meetings that included substantial treatment of our extinction analysis. It was mostly from these accounts that physicists, astrophysicists and geologists heard of our work, and this led to many requests for more information and for preprints. We were unable to send preprints until early October when the manuscript was complete and ready for submission to *Proceedings of the National Academy of Sciences*.

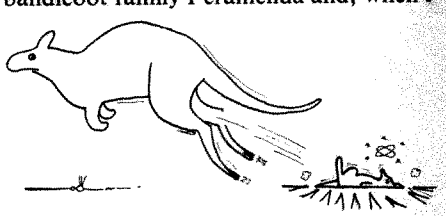
In view of the foregoing, we do not think that the situation leading to the publication of the five papers on extinction in the 19 April issue of *Nature* fits the classic description of scientific communication-by-preprint. Notice of the research results (including the Flagstaff abstract) was available to all readers of *Science* and *Science News* and to all participants at the Flagstaff meeting. Preprints were sent out only later and then were largely limited to those requesting them. Of course, no preprints were released to the press.

We hope that the publication of this letter will clear up any confusion over this curious incident.

DAVID M. RAUP
J. JOHN SEPKOSKI JR
Department of Geophysical Sciences, University of Chicago, Chicago, Illinois 60637, USA

Directional pouches

SIR — I was fascinated by the photographs of a gaping thylacine ("Tasmanian tiger") in the series from Jon Darius's *Beyond Vision* (*Nature* **307**, 411; 1984). However, among the curious features of the thylacine, the rear-opening pouch is by no means unique. According to Walker's *Mammals of the World* (1964), the posteriorly opening pouch is typical of the bandicoot family Peramelida and, when



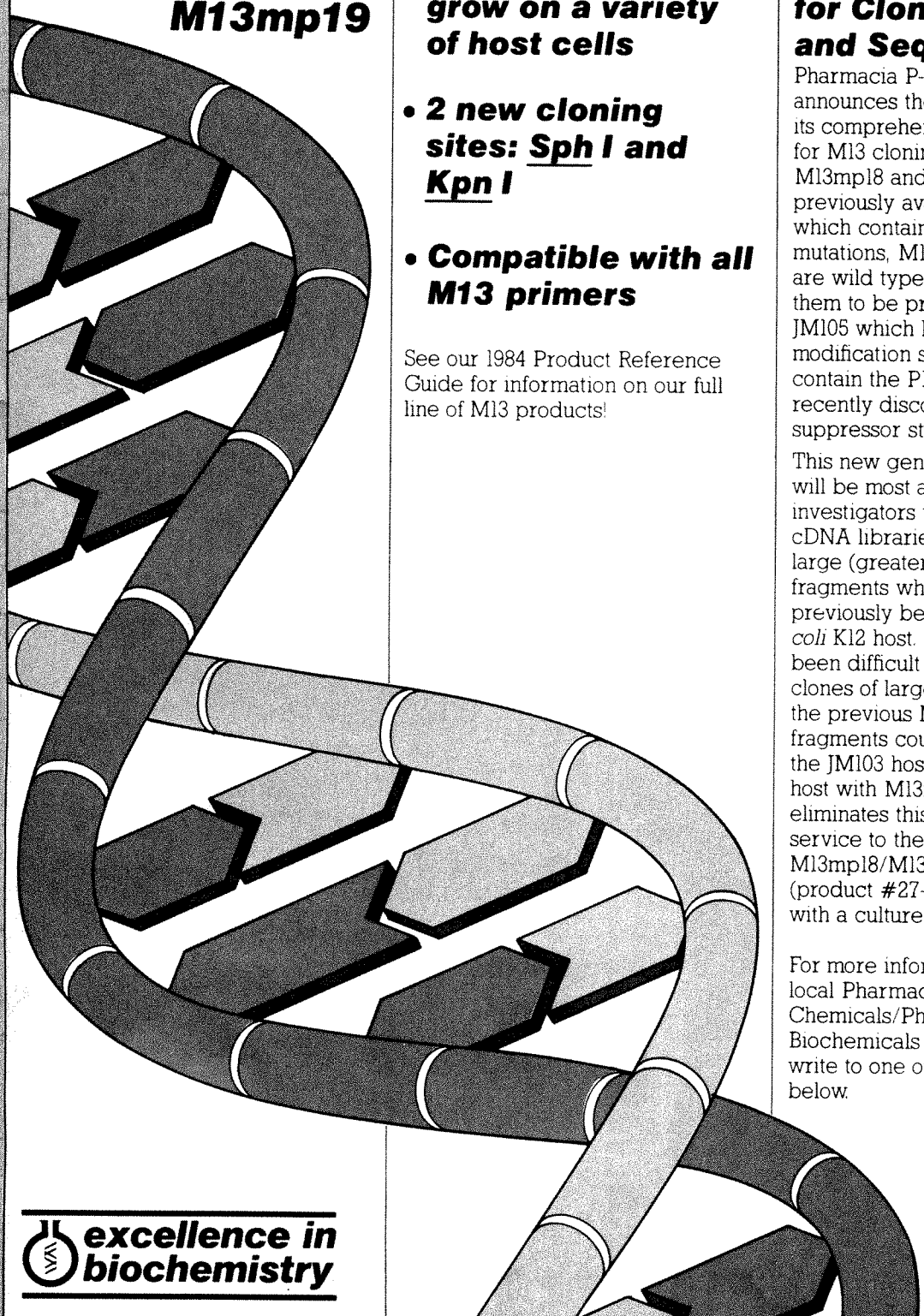
pouch is present, of the family Dasyuridat. The pouch also opens backwards in the koala *Phasolarctos*, the wombat *Phascolomis* and the marsupial mole *Notoryctes*.

I presume that in most of the quadrupedal marsupials, as in the thylacine, young in a front-opening pouch might be liable to damage by undergrowth, or in the mole, from the pouch filling with soil. Only in the bipedal macropods would a backward — and hence downward — opening pouch have obvious disadvantages.

JEREMY H. MARSHALL
St John's College, University of Oxford, Oxford OX1 3JP, UK

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Scientific Programme

		SESSION A	SESSION B		SESSION C		SESSION D
MON 9	AM	Immunocytochemistry and related new Localisation Methods	Microscopy of Interfacial Structures (HREM)	I	Specimen Preparation (Biological)		X-Ray Microscopy
	PM			II	Complementary LM & EM		X-Ray Microscopy
TUE 10	AM	Immunocytochemistry and related new Localisation Methods	Microscopy of Interfacial Structures (III—Semi-Conductors) (IV—Coating)	III	Application of LM Techniques—Materials	I	TEM/STEM State of the Art
	PM			IV	Application of LM Techniques—Life Science	II	TEM/STEM State of the Art
WED 11	AM	Environmental and Forensic Microscopy	Tutorials in Modern Aspects of Microscopy (SEM/LM)		Microscopical Measurement (LM)		Image-Recording and Computer-aided collection of Data
	PM	Archaeological Microscopy		I	Acoustic Microscopy Acoustic Microscopy		Image Recording and Computer-a collection of D
THU 12	AM	In Situ Hybridisation	Histochemistry of the Eye			I	Cryotechniq
	PM	Proteases & Peptides	SEM Optimisation— The Instrument	II	Biological X-Ray Microanalysis Biological X-Ray Microanalysis	II	Cryotechni
FRI 13	AM	Proteases & Peptides	SEM Optimisation— Preparation Techniques				
	PM		SEM Optimisation— Information from the Image	III			

The detailed content of each session is available on request. Booking forms giving full details of accommodation are also available. It is possible to register either for the full week, by day or for a half day.

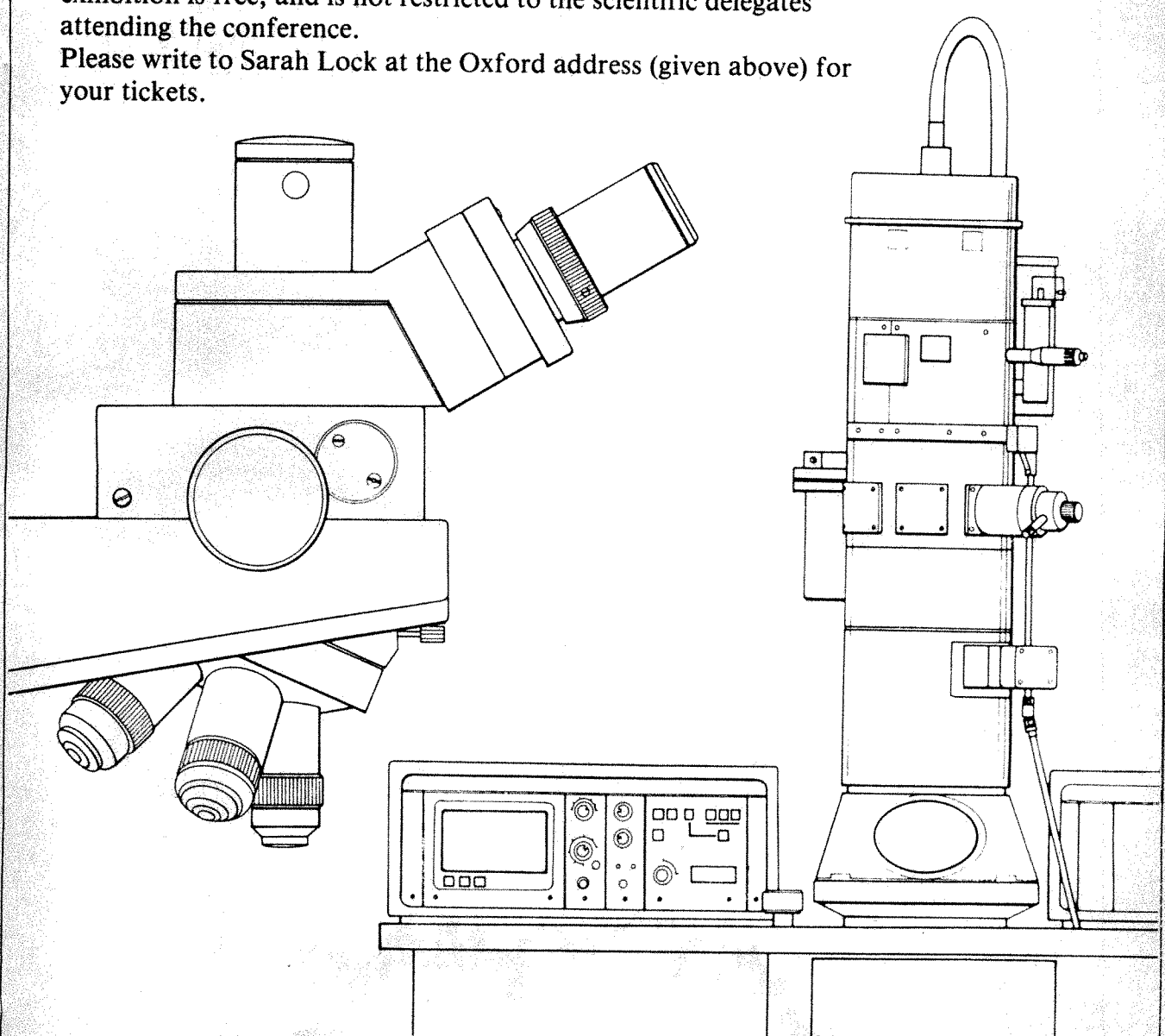
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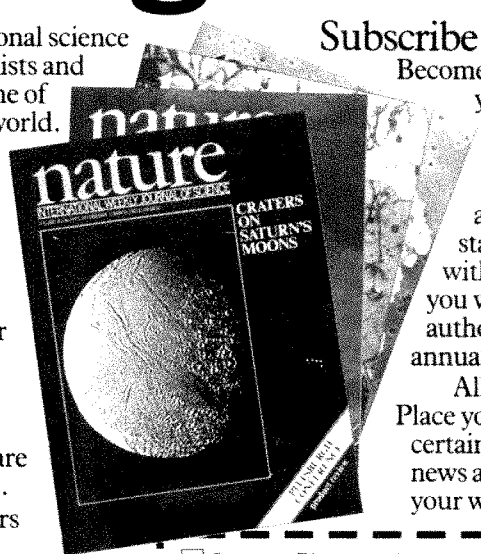
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Anatomy of a pressure group

from Stephen Budiansky

Mr Jeremy Rifkin's assault on genetic manipulation seems to be succeeding. The danger is that he will not be taken seriously.

IF it were not for the prominent names that keep popping up on his manifestos, people would probably ignore Jeremy Rifkin, the self-styled social crusader who hopes to save mankind from genetic engineering. Rifkin's remarkable knack at canvassing for credibility is once again in evidence, in the case now before Judge John Sirica in Washington, DC in which Rifkin is seeking to halt the first field trials of genetically-engineered bacteria. And it is hard to dismiss one who can wave affidavits from respected scientists who appear to support his claims that serious unfathomed risks are lurking behind these unprecedented experiments.

Combining frequent references to his gallery of the famous with a stream of eminently quotable apocalyptica, Rifkin has succeeded in conjuring an image of genuine controversy. The fact that scientific opinion runs virtually unanimously against him and that his "support" from the famous proves time and again to be far less than meets the eye tells much about public relations on complex scientific issues and about the political naivete of too many scientists.

Crop protection

The case now before Judge Sirica deals with an experiment approved by the Recombinant DNA Advisory Committee (RAC) in April 1983 in a decision remarkable at the time only for its seeming total lack of controversy. Two University of California researchers, Steven Lindow and Nikolas Panopoulos, were hoping to demonstrate the ability of genetically-altered bacteria to protect field crops against frost damage. Under natural conditions, certain strains of wild bacteria that colonize the leaves excrete a protein that serves as a nucleation site for ice formation at a temperature slightly below freezing. If it were not for this protein, temperatures could drop as low as eight degrees Fahrenheit below freezing without frost actually forming. Lindow and Panopoulos believe that by displacing these wild bacteria with laboratory bacteria that have been genetically altered so that they cannot produce the protein, the growing season could be extended by several weeks in the spring and fall. A single gene is responsible for the protein, making this an obvious candidate for genetic engineering.

Although the proposal by Lindow and Panopoulos was RAC's first request for permission to release a genetically engineered microbe outside the laboratory, it seemed to raise no real safety questions. The researchers had already performed identical experiments with chemically-mutated bacteria — experiments which, if anything, were more dangerous yet which did not require RAC's approval as they did not involve recombinant DNA techniques. The field tests with the engineered bacteria were to be done at a single site, geographically isolated from California's citrus and fruit crops, and the bacteria were to be pre-screened in a greenhouse against any tendency to damage plants. After hearing the report of an outside consultant, Ann Vidaver, a plant pathologist at the University of Nebraska, and noting that the researchers had altered their experimental proposal in response to minor criticisms made earlier by RAC. RAC unanimously approved the experiments.

The experiments were to begin in November 1983. On 14 September, Rifkin filed suit in US District Court, arguing that RAC's approval was invalid because no environmental impact statement had been filed, a requirement for all federal actions having a "significant" effect on the environment. Rifkin also threatened to file for a temporary injunction against the University of California to halt the field trials pending a ruling on his lawsuit.

Lindow and Panopoulos postponed their experiments until this spring, by which time, they hoped, Sirica would have disposed of the matter. Last week, following Lindow's declared intention to proceed with the experiment later this month, Sirica granted Rifkin a preliminary injunction, halting the experiments until he could hear the full case (see p. 296).

Suing the government, of course, has become standard practice in the environmental movement. Key environmental legislation of the 1970s granted citizens an explicit right to actions; other legislation allows those who sue the government in the public interest to collect attorneys' fees if they win. This mechanism has proved a valuable remedy for foot-dragging by the government and has served to ensure that government agencies follow proper procedures.

But suing the government is also a great attention-getter. In the present case, Rifkin has not taken advantage of repeated opportunities for public participation in RAC's deliberations (saying he was out of town on those days). The nuisance-to-substance ratio of the lawsuit is high — at best, a final decision in Rifkin's favour will require only that RAC go through the formality of producing an Environmental Impact Statement for deliberate release experiments; after that, it can approve whatever experiments it wants without further judicial review. But a barrage of hyperbolic press releases accompany the suit, calling RAC "grossly irresponsible" and accusing it of "reckless disregard for the protection of the environment". In an interview, Rifkin said that the release of genetically-engineered organisms to the environment may "deplete the life-support system of the planet".

Scientists' support

Such statements, of course, make good copy. They also make scientists groan. But Rifkin's ace is the collection of experts he has corralled. "You can dismiss me", he says when challenged about the soundness of his more extreme comments, "but it's very hard to dismiss Peter Raven".

Peter Raven, director of the Missouri Botanical Garden, professor at Washington University and member of the National Academy of Sciences, is probably the most prominent name on Rifkin's list of scientific supporters. His affidavit, like the others, speaks mainly in generalities about the dangers of introducing new species into established ecosystems, and seems to betray an ignorance of the details of the Lindow experiments.

Remarkably, Raven freely admits that he had seen neither the detailed experimental proposals nor RAC's minutes before filing his affidavit. And he said, "I'm not criticizing RAC at all. I haven't even got a position more than to say we need visible discussion." Asked why he did not use RAC's public meeting or the nearly year-long opportunity to file comments with RAC, he said he had "no way of knowing" how he could participate in RAC's deliberations. "Nor was my opinion sought." Raven said the burden must be on RAC to reassure the ecological community that adequate scrutiny was given to the proposal.

Eugene Odum, an ecologist at the University of Georgia, also filed an affidavit. He said he was voicing his concern over the lack of proper procedures for evaluating deliberate-release experiments, not the Lindow experiment itself. "I would doubt that the experiment itself is dangerous", he said, "but it could establish a precedent that's very dangerous."

Yet the procedures are already in place for evaluating deliberate-release experiments. Maxine Singer, a researcher at the National Institutes of Health (NIH) who has been a strong voice for caution since 1973, says that tactics such as Rifkin's lawsuit are unlikely to bring about a more thorough scrutiny of genetic engineering research. "The evidence is there that RAC asks difficult questions and is not about to give permission at the snap of their fingers", she says. And everyone, from Jeremy Rifkin to the biotechnology industry, agrees that, although compliance with RAC's decisions is technically voluntary for private industry, no company would dare proceed without RAC approval, since a company's failure to abide by the guidelines and RAC's decisions would be very damaging evidence of negligence if the company were sued for damages arising from an experiment. And Singer doubts whether the Environmental Protection Agency could command the political clout and scientific respect of RAC.

David Baltimore of Massachusetts Institute of Technology speaks for many when he says that "there are aspects of the direct-release experiments that raise problems that RAC previously didn't have to deal with. But I don't think it's helpful to make generalizations. You have to think carefully about each case. You can't do it on the basis of what you hear from Jeremy Rifkin or what you read in the *New York Times*." And he warns of the dangers of a backlash against Rifkin's tactics: "The continual opposition to recombinant DNA activities has made the scientist community leery of raising even those concerns they feel are legitimate, because they can so easily be taken advantage of. And that's a terrible climate. But it's the inevitable consequence of politicization."

Endangered humanity

This lawsuit is not the first occasion that Rifkin has pulled credibility out of a hat. Last June, after canvassing religious leaders across the country, Rifkin produced a petition calling for an "immediate" ban on engineering of the human germ line. Joining the call were 21 Catholic bishops, leaders of most major protestant sects in the United States, several Jewish leaders, the Moral Majority's Jerry Falwell and television preacher Pat Robertson. A "theological letter" accompanying the position provided the apocalyptic rhetoric — calling engineering of genetic traits "a fundamental threat to the preservation of the human species as we know it". The letter claimed that "it will soon be possible to carry out such procedures on humans, and argued that even the elimination of defective genes — such as those responsible for sickle-cell anaemia — would dangerously narrow the human gene pool and "might ultimately lead to the extinction of the human race".

Rifkin concluded by equating genetic engineering with eugenics ("the inseparable ethical wing of the Age of Biotechnology") and by calling upon society to oppose human genetic engineering "with the same courage and conviction as we now oppose the threat of nuclear extinction".

Once again, there was less than met the eye. The religious leaders who appeared at Rifkin's New York City press conference were careful to disavow the "theological" letter; they had signed only the much more moderately-worded petition. Subsequent interviews revealed that many of the signatories had minor or even major reservations about the petition as well. Some said they did not oppose treatment of disease, and had signed only to express their agreement with Rifkin's warnings about the misuse of genetic engineering for eugenics. Many reaffirmed their support for somatic-cell gene therapy for the relief of human suffering and the treatment of disease.

Once again, Rifkin may also have alienated those who might otherwise have been his natural allies. Alexander Capron, who directed the President's Commission for the study of Ethical Problems in Biomedical and Behavioral Research — which, until its demise last year, produced a series of carefully argued and widely praised studies of ethical issues in medicine, including genetic engineering — called the petition a "knuckleheaded, broadside" attack that swept aside a host of subtle ethical dilemmas in favour of polemics.

John Fletcher, an Episcopal minister who, as assistant to the director of NIH for bioethics, has thought long and hard about these issues, was also irked by the petition. He wrote to Bishop John Allin, the presiding bishop of the Episcopal church in the United States and a signatory of the petition, criticizing its "apocalyptic" tone and its failure to take into account the "thinking that had already been done". Fletcher said he wishes the signatories had "asked for help to become informed on the body of debate that already existed. It is interesting that the President's Commission report [*Splicing Life*, produced at the request of the major religious organizations in the United States] had existed for a year, and many [of the petition's signers] had not seen it."

Fletcher, significantly, is one of those who have urged caution in proceeding with germ-line alterations, arguing that society at present lacks the necessary ethical and moral system to deal with the consequences of man's engineering his own heredity. "But I am in favour of keeping an open mind", he said.

Others point out that "this is not something that is happening suddenly", as Dr French Anderson, chief of the Laboratory of Molecular Hematology at NIH and a leading researcher on genetic blood disorders, put it. Anderson says although the genes necessary for treating some 20 inherited disorders have been isolated, "we can't get them into the target cells, we can't regulate them properly and we have no way of assessing their safety" in humans. When it becomes a scientific possibility, the real ethical questions may have little to do with somatic versus germ-line alterations but — as pointed out by the President's Commission report — whether the alteration is reversible (should some unforeseen danger later arise), whether it is aimed at correcting recognized genetic disorder or at "perfecting" humans.

Regulation

In the United States, the regulatory mechanism is also already in place to assure that germ-line manipulation is not rushed into without considerable deliberation. Experiments on human subjects will have to be cleared by a local Institutional Review Board charged with protecting human subjects; the board must include at least one non-scientist and at least one scientist not affiliated with the institution. Human gene therapy trials would also have to be cleared by a similar local panel, the Institutional Biosafety Committee, charged with overseeing recombinant DNA research. The full RAC would then have to give its approval and, before it could be applied routinely in clinical practice, the Food and Drug Administration would have to approve its safety and effectiveness.

The case of Martin Cline, a researcher at the University of California, Los Angeles, who performed unauthorized (and unsuccessful) gene therapy experiments on two patients suffering from beta-thalassaemia, a fatal genetic blood disorder, was ample proof that these regulations are not merely cosmetic. When Cline's experiments became known he was stripped of federal research grants and found his research career effectively ruined.

Just as with his lawsuit, the net result of Rifkin's petition may have been polarization — and a backlash against sensible proposals. Congress, for example, is seriously contemplating the establishment of an advisory body, along the lines of the President's Commission, to monitor developments in human genetic engineering. The concept, championed by Representative Albert Gore, is strongly backed by the President's Commission itself, Capron, Fletcher and many others concerned about the ethical and religious implications of genetic engineering.

RAC, the other logical body to exercise oversight on human genetic engineering, has also recently proposed expanding its membership beyond researchers and physicians to include ethicists.

But, as Capron says, there is a danger that those in research and industry who might otherwise support these proposals may come to see them as "part of the Rifkin attack". The danger is that the very concerns that Rifkin claims to represent may be swept aside, resulting in an all-out political battle between two polarized camps. □

Chaos — a model for the outbreak of war

from Alvin M. Saperstein

The chaos as formulated for physical dynamic systems is applied to a very simple nonlinear model of the arms race. The transition, from stability to instability, from arms race to war, could be analogous to the transition from a laminar to a turbulent (or chaotic) flow.

SIMPLE mathematical models of the arms race have aroused interest among academic physicists[1]. Two common models are the dynamic model of Richardson[2,3] and the static inequality model of Kaye[4,5]. Both are deterministic: given the model and its parameters, a reasonable estimate of present conditions allows prediction of the future outcome of the arms race. Here I offer the beginnings of another model based on recent advances in the understanding of chaotic motions of dynamical systems[6,7]. I suggest that war be viewed as a breakdown in predictability: a situation in which small perturbations of initial conditions, such as malfunctions of early-warning radar systems or irrational acts of individuals disobeying orders, lead to large unforeseen changes in the solutions to the dynamical equations of the model. There is no way then to predict the effect of the actions of any participant — analyst, planner, statesman or general — with any certainty. Certainly war, between roughly equivalent parties, has often been characterized by uncertainty in outcome[8]; nations which start wars in great confidence very often end defeated[9]. Typically, in the period immediately before the outbreak of a war the nations attempt desperately to claw back as they slide over the precipice, although they by this time already have lost control of their own destinies[10]: control and predictability are lost.

There is a current view that entrance into war, and the war itself are deterministic — escalation, up or down, nuclear or otherwise, can be controlled[11,12]. But most historical evidence and contemporary analysis indicates otherwise: predictability and control are lost at the threshold between peace and war[13], just as they are lost when a fluid makes the transition from laminar to turbulent flow.

Here I sketch the meaning and development of chaos in nonlinear dynamical systems and apply these concepts to a simple nonlinear arms race between two nations. Both the model and the illustrative parameters are admittedly over simple and do not as yet apply to the real world, but I hope that the concept of 'chaos', as used herein has a bearing on the present world situation. I believe that nonlinear dynamical models, with their capability for both predictable and non-predictable orbit through the phase space of international competition, are necessary for realistic understanding of the present arms race.

Deterministic models patterned after those used successfully in physics have previously been applied to the description of competition in economics and biology[14]. Because these models may also have a significant nonlinear component, it follows that 'chaos' may also result from these competitions; some examples have been reviewed by May[7].

Richardson's model for the arms race between two competing states consists of a pair of coupled first-order linear differential equations. The independent variable is time and the dependent variables are measures of the devotion of the two societies to hostilities — expenditure on arms, percentage of population in the military, and so on. The coupling terms, the off-diagonal elements of the matrix of the system of differential equations, represent the reaction of each nation to the challenge of the other: thus the rate of change of the armaments of nation A is assumed proportional to the total armaments of nation B and vice versa. The diagonal terms of the system represent the intrinsic reaction of each nation to its own armaments; for example, a large military establishment might successfully press for further increases in military expenditure, or the rest of society resist increases in the share of resources going to the military.

The solutions of such a model system are simple linear combinations of exponentials in time. The exponents are determined by the model parameters whereas the coefficients of the linear combinations are uniquely determined by the initial conditions. Small changes in the initial conditions imply small changes in the coefficients, so that two solutions which start very close together will always remain comparatively close together. This is the sense in which the behaviour over time of a dynamical system is predictable. Knowledge of initial conditions is always uncertain, which means that a set of orbits, or a 'flow', is allowed by any practical model. Since all orbits in the Richardson model flow remain close to each other, practical predictions remain possible.

If the model parameters are such that the exponents are negative, the solutions damp down to an equilibrium value no matter how starting conditions are chosen. Such a case is 'stable'. If the model parameters imply positive exponents, the flow departs exponentially from any starting conditions and the armaments of both parties increase exponentially. This situation is 'unstable' and may imply war or the certainty of eventual war.

A peacemaker in Richardson's model must alter the parameters of the competition so that only negative exponents are possible. If he accomplishes that, he can successfully predict peace (or at least inventories of the tools of war which do not grow) no matter what the starting configurations are. Conversely, if the parameters of the model lead to instability, the generals can know what to expect, given reasonable intelligence of the starting configuration.

The Richardson model is dynamic, relating changes in the configuration to a present configuration, and stability or instability to the kinds of change possible. By comparison, the Kaye model of bilateral strategic deterrence[4,5] is static, dealing with the two parties' perceptions of their situation. The variables are the numbers of nuclear warheads each is capable of firing at the other, the model parameters are the dependability, accuracy and destructive capabilities of these warheads. There is no time parameter or variable.

Given one set of inequalities between the variables, one side can punish the other without undue loss. That side is, therefore, secure and can afford to relax; the other side perceives no such security. Given another range of inequalities between the same variables, both sides are assured that they can inflict unacceptable damage upon their opponents even after suffering the worst first-strike devastation their opponent is capable of delivering; both sides perceive 'mutually assured destruction' so have no incentive either to launch a first strike or radically to change their present state of armament. This represents 'stability' or 'stable deterrence' in the Kaye model. Finally, the range of inequalities remaining includes the situation where each side can destroy the other so that the side that strikes first can prevent its victim from retaliating: each side has an incentive to strike first or radically to increase its armaments. The situation is unstable or is said to represent 'unstable deterrence'.

The Kaye model is described by a Cartesian plot in which the two orthogonal axes represent the number of warheads or launch vehicles available to each of the two sides. The inequalities are represented by different regions of the plot. For example, in Fig. 1, adapted from ref. 5, the number of

launch vehicles possessed by side A, N_A, is plotted against N_B, the corresponding number for the opposing side B. In the region designated DA, A is dominant; it can punish B with a successful disarming first strike without fear of severe loss to itself. Similarly B is dominant in region DB. Region MAD is the stable region of mutually assured destruction whereas FS is the unstable deterrence region in which each side has the incentive to strike first. The remaining region, IN, represents that in which neither side has sufficient warheads to do conclusive damage to the other. Thus there are stable and nonstable areas which, topologically are simply connected and have simple boundary curves. Except for the boundary curves themselves, nearby points in the plot belong to the same region. Thus small uncertainties about the actual numbers need not lead to changes in the perception of stability or instability. In this sense, the static model also permits practical predictions.

In physics, we are accustomed to assuming predictability based on Newton's equations of motion. Given the forces, specific initial conditions lead to specific well-defined orbits in an appropriate phase or coordinate space. Similar concepts lead to the nonlinear equations of fluid dynamics. Since actual fluid motions exhibit both laminar and turbulent flows, we must accept that the solutions of the equations of fluid dynamics may represent either laminar or turbulent character and that the nature of the flow changes from laminar to turbulent as some parameter or combination of parameters increases through some critical value[15].

Laminar flow implies predictable behaviour: streamlines which start off near to each other remain near to each other. Knowledge of the motion at one point in the flow at one point in time implies knowledge of the motion at neighbouring points in space and time. Turbulence, by contrast, is chaotic: knowledge of the motion at one location at one time says nothing about the motion at nearby points at the same time or at the same point at later times: prediction is fundamentally impossible. Hence the same mathematical model (the same set of equations) allows predictable behaviour in some regions of a model parameter, and unpredictable behaviour in others. This breakdown of predictability in certain systems seems a good characterization of war in those systems.

Civil life presupposes a major component of predictability. The formulation and carrying out of policy requires a reasonable ability to predict the future given some knowledge of the present. The same requirement for predictability holds for the relationships between nations, whether they are engaged in normal international competition or in a hostile arms race. But the history of nations is full of examples of a loss of control, by one or more governments, as they make the transition from peace to war. Thus any single model of an arms race between nations should include regions of predictable behaviour, regions of chaos and transitions between such regions.

As a simple model of the transition from predictability to unpredictability consider the flow represented by the sequence of mappings[6]:

$$x_{n+1} = 4bx_n(1-x_n) = F_b(x_n) \qquad (1)$$

where $0 < x_n < 1$, $n = 0, 1, 2 \ldots$ and $0 < b < 1$. A specific orbit is the succession of values $x_0, x_1, x_2 \ldots$ starting from a specific value of x_0. A flow consists of a set of orbits, starting from a set of neighbouring values of x_0. The flow is predictable if its constituent orbits remain near each other; it is unpredictable (chaotic) if they drift apart exponentially: if, given any x_0 and x_0 , the difference between x_n *and* x_n diverges exponentially as $n \to \infty$. No matter how small the subinterval ε is from which a chaotic flow starts, it soon fills the entire interval (0,1); no matter how accurately the starting value x_0 is measured if the motion is chaotic knowledge of the position of x_n is soon lost — it is distributed with equal probability throughout the allowed region (0,1). Prediction is impossible for a chaotic flow: the flow determined by (1) above changes from predictable to unpredictable as b increases through the critical value $b_c = 0.892$.

Let x^* be a fixed point of a flow, F, defined by the equation

$$x^* = F(x^*) \qquad (2)$$

Then x^* is a stable fixed point if

$$F'(x^*) = \left| \frac{d}{dx} F(x = x^*) \right| < 1 \qquad (3)$$

If x^* is a stable fixed point, it is easy to see[6] that all orbits, no matter where started, tend to x^* as $n \to \infty$. Thus given such an x^*, perfect prediction is possible: wait long enough and any flow converges to x^*. It is easy to show that $x_b^* = 0$ is the only stable fixed point of F_b for $b < \frac{1}{4}$. For $\frac{1}{4} < b < \frac{3}{4}$, the only such point is $x_b^* = 1 - (4b)^{-1}$. And for $b > b_1 = \frac{3}{4}$, there is no stable fixed point of $F_b(x)$.

Consider now the mapping $F_b^{(2)}(x) = F_b(F_b(x))$ and its fixed points x_b^{**}. If x_b^{**} is not a fixed point of F_b, it follows that

$$F_b(x_b^{**}) = \bar{x} \text{ and } F_b(\bar{x}) = x_b^{**}$$

where $\bar{x}$ does not equal x_b^{**}, (note that x is also a fixed point of $F_b^{(2)}$). Thus $x_{n+2} = x_n$, $x_{n+1} \neq x_n$, if $x_n = x_b^{**}$. It follows that the flow F_b oscillates back and forth periodically between pairs of stable fixed points of $F_b^{(2)}$, if these exist while those of F_b do not. Such a situation occurs for $\frac{3}{4} = b_1 < b < b_2 = 0.862 \ldots$ Again, prediction is possible, though more complex.

For $b > b_2$, the fixed points of $F_b^{(2)}$ are no longer stable, so that it is no longer possible to say that the flow converges to a periodic oscillation between two points. But there is now an interval $b_2 < b < b_4$ for which the fixed points of $F_b^{(4)}(x) = F_b^{(2)}(F_b^{(2)}(x))$ are stable. The flow now converges to a periodic oscillation between four points. Prediction is still possible though even more complicated. As b increases further, the fixed points of $F_b^{(4)}$ become unstable, the four-point periodic motion disappears and the flow now converges to an eight-point cycle, which then becomes a 16-point cycle and so on.

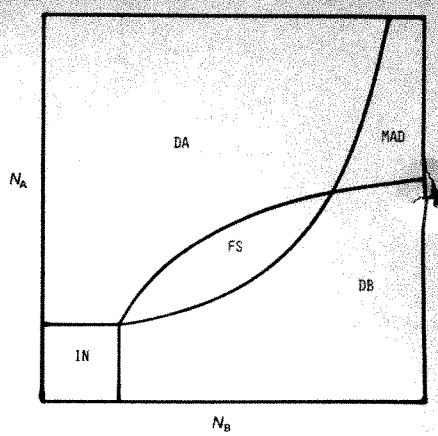


Fig.1 A strategic balance diagram (Kaye plot) showing regions of stability and instability.

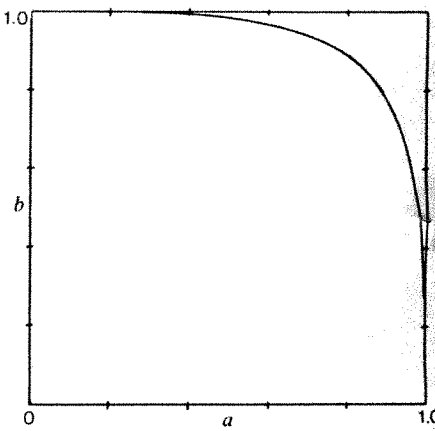


Fig.2 A curve representing $f_4(a,b) = 0$ (see text), taken to be a reasonable approximation to the critical curve $f_c(a,b) = 0$ separating the region of predictability from that of chaos. Data courtesy of Larry Dishman, Wayne State University.

There is thus a sequence of periodic oscillations of ever-increasing complexity corresponding to a sequence of transition values b_i with an accumulation point at $b_c = 0.892 \ldots$ Beyond this value lies chaos: orbits do not have definite end points, initially neighbouring orbits diverge exponentially, any flow soon fills the entire interval and prediction is impossible. In this simple model, b_c corresponds to the critical Reynold's number which governs the transition to fully turbulent fluid flow.

A bilateral arms race between two competing countries X and Y is assumed to occur in stages designated by an integer indicator n; successive values of n may indicate successive years, successive budget cycles and the like. (Thus n is a discrete version of the independent variable, time, in the Richardson model.) The dependent variables x_n, y_n represent the fraction of the two nations' resources devoted to armaments: $0 < x_n, y_n < 1$. A nation's armaments at the next stage depend upon its perception of its opponent's armaments at the present stage; I will assume x_{n+1} to be proportional to y_n and conversely.

Table 1 Ratio of national military expenditure to GNP

	France	Germany	Italy	UK	USSR
1934	0.0276	0.0104	0.0443	0.0202	0.0501
1935	0.0293	0.0125	0.0461	0.0240	0.0552
1936	0.0194		0.0298	0.0296	0.0781
1937	0.0248		0.0359	0.0454	0.0947

But the perceived threat also depends upon how much of the opponent's resources remain potentially convertible to armaments at a future stage. If all of the opponent's substance is devoted to arms, there is no need to be concerned about a future increase in threat (assuming fixed total resources). Hence I also assume x_{n+1} to be proportional to $1-y_n$ and conversely. My simple arms race model thus consists of the pair of sequences of mappings:

$$x_{n+1} = 4a\,y_n(1-y_n) = F_a(y_n) \quad (4)$$

$$y_{n+1} = 4b\,x_n(1-x_n) = F_b(x_n) \quad (5)$$

in which $0 < a, b < 1$. If the values of a and b are such that the flows are predictable the race will be said to be stable. Each party will know what the future portends and can plan accordingly. If the parameters product a chaotic flow, the race is unstable and neither can know what the future will bring.

Analysis of the model with two parameters and two variables represented by equations (4) and (5) is similar to that of equation (1). Fixed points are characteristic of the mapping of a variable onto itself. Thus in this case the appropriate sequences of mappings are not F_a and F_b but, instead,

$$F_{ab}(x) = F_a(F_b(x)) \text{ and } F_{ba}(y) = F_b(F_a(y)) \quad (6)$$

Fixed points of these sequences imply $x_{n+2} = x_n$ and $y_{n+2} = y_n$ and are again stable if the absolute magnitude of the derivative is less than unity. It is easy to show that, for each fixed point x_i^* of F_{ab}, there is a corresponding fixed point y_i^* of F_{ba}; with $y_i^* = F_b(x_i^*)$ and $x_i^* = F_a(y_i^*)$. Further, if one of the pair of fixed points is stable, then so is the other since the two slopes are equal. A similar relationship connects the fixed points of $F_{ab}^{(2)} = F_{ab}(F_{ab})$ and $F_{ba}^{(2)} = F_{ba}(F_{ba})$, and so on. Hence, there cannot be predictability in one of the variables and unpredictability in the other. Both parties to the arms race will be at war or at peace simultaneously, which corresponds with commonsense.

Again, as the values of a and b are changed, there is a sequence of flows in which first the fixed points of F_{ab} and F_{ba} are stable; then these fixed points become unstable while those of $F_{ab}^{(2)}$ and $F_{ba}^{(2)}$ remain stable; these then become unstable while the fixed points of $F_{ab}^{(4)} = F_{ab}^{(2)}(F_{ab}^2)$ and $F_{ba}^{(4)} = F_{ba}^{(2)}(F_{ba}^{(2)})$ remain stable, and so on. Instead of a sequence of transition points b_i and a critical accumulation point b_c, as above, there is now a sequence of transition curves $f_i(a,b) = 0$ (symmetric in a and b) and a critical accumulation curve $f_c(a,b) = 0$ beyond which the two flows are chaotic.

The actual transition values, a_i, b_i, for the model of equations (4) and (5) have been estimated graphically. Plots were made, for a sequence of values of a and b, of $F_{ab}(x)$, $F_{ab}^{(2)}(x)$, $F_{ab}^{(4)}(x)$. Intersections with the straight line $F(x) = x$ were determined and the slopes F_{ab}, $F_{ab}^{(2)}$, $F_{ab}^{(4)}$ were measured by eye. A transition was judged to have occurred when the absolute magnitude of one of the slopes exceeded unity. Given the crudeness of these measurements, the critical accumulation curve is assumed to be indistinguishable from the curve $f_4(a,b) = 0$ which determines the transition of $F_{ab}^{(4)}$ from stability to instability and which is sketched in Fig.2. Chaos, and hence war, occurs for those values of the model parameters a, b lying above and to the right of the curve.

It is tempting to apply this model to previous and present arms races even though the data are difficult to obtain and the model is over-simple. Some data are available for the period immmediately preceding the Second World War[12], although the figures are questionable. Nevertheless, it is possible to assign values for the ratio of national military expenditure[16] to gross national product (GNP)[17] (Table 1).

The values in Table 1 are x_0, y_0, x_y, and y_1 (subscripts 0 and 1 designate the earlier and later of the two years used) to be substituted into equations (4) and (5) to obtain parameters a and b for pairs of competing countries in the European arms race during the 1930s (Table 2).

Table 2 The parameters a and b

		a	b
France–Germany	(1934–35)	0.712	0.116
France–Italy	(1936–37)	0.214	0.472
UK–Germany	(1934–35)	0.582	0.158
UK–Italy	(1934–35)	0.142	0.582
USSR–Germany	(1934–35)	1.34	0.0657
USSR–Italy	(1936-37)	0.819	0.125

Thus the German–Soviet arms race was in the chaotic region, and the French–German and Italian–Soviet races were clearly close to the transition region. We all know that the Second World War broke out shortly thereafter, but it is difficult to give much credibility to this *post hoc* calculation because the model is purely bilateral.

Turning to the present-day arms race between the United States and the Soviet Union, I assume for the sake of illustration that 7 per cent of the US GNP is devoted to military and associated expenses, so that $x_0 \approx 0.07$. Assuming that Soviet armament expenditure is comparable with that of the United States and that Soviet GNP is roughly half that of the United States, it follows that y_0 0.14. Given the crudeness of the numbers and the model, it is reasonable to assume $x_1 \approx x_0, y_1 \approx y_0$ from which it follows that $a \approx 0.15$, $b \approx 0.54$. Thus the Soviet–American arms race at present lies within the stability range of Fig.2. Instability develops when b (or a) approaches unity; this would occur if y_0/x_0 increased from two to between three and four. Given the crudeness of the model and choice of parameters, no comfort can be drawn from this last statement. A more realistic model may require more than one dependent variable for each side. With such additional variables the threshold to chaos may be lower.

The crude model presented here is not a credible representation of world affairs. At the very least, it should be expanded to include the equivalent of Richardson's diagonal terms: the direct affect of x_n upon x_{n+1}, of y_n upon y_{n+1}. Also required is a careful determination of the model parameters, the a and b of the off-diagonal terms as well as corresponding coefficients for the diagonal terms[18,19]. This latter task would require the accumulation of data for arms expenditures and GNP over a sequence of several years, a task made difficult by the lack of Soviet data comparable in definition and quality with the generally available US data[20,21]. There is also the problem of the definition of military expenditure in today's high technology world.

Thus any realistic model of a two-party arms race will most probably include nonlinear terms as a result of which there may be a transition from a predictive state of affairs to chaos. The creation and analysis of such chaos-predicting models and the practical applications of the insights they provide can contribute to avoidance of such chaos in real life. □

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Particle people compile data

The latest compilation of particle physics data is a monument not merely to those who discover new particles but also to those who have improved the accuracy of what is known.

THE complaint that there are simply too many material particles for any of them to justify the old adjective "fundamental" may seem, on the face of things, to be fully borne out by the latest compilation of properties by the Particle Data Group, a collaboration of 23 physicists which is an international outgrowth of the old Berkeley Particle Data Group on which responsibility for a critical review of available data used to rest. The new compilation, based on data which had become available by the end of 1983, runs to 304 pages of the *Reviews of Modern Physics* for April this year (**56**, S1–S304; 1984) and is likely to be many people's bible for years to come. It is also a model of how data should be compiled in a fast-moving field. The moral seems to be that the job is best done by people who are themselves practitioners.

In reality, the compilers have had to break with the traditions of their predecessors and now explain that they can no longer include references to all relevant publications in high-energy physics bearing on the properties of particles. The literature, they say, is growing by about 10 per cent a year, with the result that archival material must rest where it lies, in previous compilations, the most recent of which was two years ago. Laconically, however, the new list of particles includes (under "stable particles", if you please) the measured values of the masses of the W and Z particles first discovered just over a year ago at CERN, the European high-energy physics laboratory.

The classification of these particles (along with the photon) as stable is not as eccentric as it may seem. The criterion is that particles whose decay is accomplished only by the intervention of weak nuclear forces are counted as being stable. By the same test, a whole string of unstable mesons and baryons are thrown together in the same group.

To outsiders, perhaps the most surprising feature of these listings is the comparative speed with which it seems to have been possible to refine the accuracy of the masses of recently-discovered particles. Thus, among the group of three known leptons (electrons, μ-mesons and τ-mesons, each with negative or positive electric charge), the masses of the first two are understandably known to within one part in a million (after eight and four decades respectively), but even that of the τ-meson is known to within 2 parts in 1,000 after less than a single decade. Among particle physicists, it must be tempting to hope for a further order of magnitude in the accuracy with which the mass of each particle is known with the passage of each decade. But this can only be a rule of thumb; the estimated mass of the first strange meson (K), discovered more than thirty years ago, is hardly more precise (at one part in 50,000) than that of the J/ψ, just over a decade old and the first experimental proof that charmed quarks do indeed exist.

In the circumstances, it is something of a surprise that while the mass of the neutron is known (as it should be after all this time) to better than one part in a million, the lifetime of this particle against radioactive decay (into a proton, a negative electron and an antineutrino) is known only to within 2 per cent or thereabouts.

This is not, of course, the scandal that it may seem to be. The experimental difficulties of anything like a direct measurement of a decay whose products are among the most common particles in the real world, and — worse still — themselves stable, are bound to be formidable. But the practical importance of knowing the decay rate of neutrons is far from negligible even in such straightforward circumstances as the production of bursts of cosmic rays from the Sun.

On the lifetime of the proton, the new compilation of particle data is also necessarily unhelpful. The authors can only quote the steadily lengthening list of largely negative searches for evidence that protons do indeed decay, as the grand unified theories uniting electromagnetic, weak and strong forces suggest they should, into, say, electrons and photons. As is well-known, the lower limits on the lifetime determined experimentally are already less than the lifetime calculated by the simplest of the theories, according to this compilation by "at least one order of magnitude". Given that the first experimental data appeared only in 1981, and that nearly a score of substantial papers on the subject have since appeared, it cannot be much more than a year before the decay of the proton is observed, or the theories put in considerable difficulty.

Another of the still-fruitless searches reported in the compilation is that for the sixth quark, labelled *top*. By now, it seems thoroughly established that the particles called mesons consist simply of a quark and an antiquark, not necessarily of the same breed. In retrospect, it is clear that the most familiar mesons (the muon and pion) and the less massive of the two strange particles discovered in the late 1940s are made only of the three quarks called *up*, *down* and *strange*. *Charm* was found at Stanford University a decade ago, and *bottom* soon afterwards, but *top* remains to be discovered.

This latest compilation of particle data has surprisingly little experimental evidence to report, all of it dating since 1981. And much of this is entirely negative — people have looked for particle resonances over a range of energies and have found nothing to suggest the existence of a *top* quark. But there is a handful of papers that appear to conclude that something happens at an energy of about 34 GeV which could be the signature of the species called toponium, a particle built from the *top* quark and its antiquark. If that is the case, the proton–antiproton collider at CERN should find *top* any day now. Some would say that the missing quark should have been found already.

Even in this dry listing, the search for free quarks is more fun — but equally fruitless. In a listing of more than 250 experimental searches (each of which is solemnly accompanied by a number giving the number of "quark events" recorded), all but a few of the corresponding entries consist simply of the digit "0". The experiments cover both searches for free quarks in accelerator experiments and those, based on the assumption that free quarks could be relatively massive and stable particles, in which people have sought to find quarks by taking vacuum cleaners to the ground beneath electrified security fences. The commentary in the compilation says that "of the several candidate cosmic ray events, one still enjoys the active advocacy of its discoverer".

Other particles have been sought and not found in great profusion. On the evidence of this listing, the magnetic monopole does not exist. From the exotic zoo of particles such as the Higgs boson (which *should* exist if only people knew where to look) and the tachyon (which entails time-reversal), the axion is singled out for special attention in this compilation, no doubt because of the recent popularity of these still unknown particles as plausible constituents of the missing mass in the Universe. But here again, for the time being at least, the experimentalists have drawn a blank.

John Maddox

Auditory physiology

Mobile maps in the brain

from John D. Pettigrew

POINT-to-point matching of an array of sensory cells to an array of central neurones produces retinotopic and tonotopic maps in the brain, which have been the stock in trade of neuroscientists tackling problems such as the control of neuronal specificity. But a neural map of auditory space cannot be generated simply by the equivalent topographical projection from the cochlea at the auditory periphery, because the cochlea represents the frequency of the tonal sound stimulus rather than its location in space. Instead an auditory space map must be computed from subtle cues provided from the effect of the head and pinna on the sound field. It therefore came as a revelation to find a 'computational map' f auditory space in the brain of the barn wl, where individual neurones are specifically tuned to different sound source locations and are arrayed so that position in the brain map is systematically related to the preferred location in space[1]. The mechanism by which such a map is generated is still being explored, but meanwhile a study of an auditory space map in the monkey's midbrain has produced another revelation, reported by Jay and Sparks on page 345 of this issue[2].

Jay and Sparks find that single neurones in the monkey superior colliculus show specificity for the azimuthal location of a sound source and are arranged topographically to form a map of sound space somewhat like that described in the owl, but with one feature that is new and surprising: the coordinate frame of the map shifts along the horizontal plane as the monkey's eyes change position. Thus, for example, a neurone which responds to a sound source located 10° to the right of the eye's fixation point when this is straight ahead will still respond to a source located 10° to the right of the fixation point when the eyes are looking 40° right, even though the head and ears have remained stationary.

The new findings have come from conceptual and technical innovation in the presentation of sound stimuli, which auditory neurophysiologists have at last moved from the ear-canal out into free-field auditory space. This move, like that made by visual physiologists in the late 1950s away from direct retinal stimulation towards the use of natual visual stimuli[3,4], avoids assumptions about the way in which complex spatial parameters of the natural stimulus are encoded; and it is proving to be as fruitful in audition as it was in vision.

One of the first free-field auditory stimulators used by auditory neurophysiologists was called Herb's Hoop, after the Caltech craftsman who built it while also working on equipment for the Viking spacecraft. Appropriately, the hoop was initiated on the same day in 1976 that the Viking lander touched down on Mars, and since that time has been responsible for a succession of important new findings, beginning with the first description of single neurones with sharply restricted receptive fields in auditory space[5] and their arrangement into a map of auditory space[1].

The finding of the computational map of auditory space in the barn owl provoked a search for a comparable map in the brain of mammals, and 'hoops' sprang up in auditory neurophysiology laboratories from Oxford to Alabama to Monash. This search is still going on in the cat, despite a string of negative findings[6,7], and difficulties have been encountered in finding any representation of auditory space in the mammalian inferior colliculus. In contrast, numerous studies have found evidence of spatial representations of a variety of stimulus dimensions, all in register, within the mammalian superior colliculus. Sound space, visual space and even somatosensory space are all mapped with the same rough topography into the superior colliculus of various mammalian species; and maps of auditory space and of the direction of infrared sources are located within the equivalent structures of the owl[8] and rattlesnake[9] respectively.

Distortions occur in the course of the generation of maps which vary according to modality. Registration between maps can therefore be a problem and considerable interest has focused on this question. Gross misregistration can be corrected developmentally, as shown in elegant experiments by the Knudsens on young barn owls wearing prisms to displace their visual fields. In this case the map of auditory space shifts to realign itself appropriately with the visual map, whose coordinate frame is unchanged (relative to both the eyes and the head within which the eyes are immobile in owls).

Despite such plasticity, smaller local areas of misregistration still occur and are puzzling if considered in purely sensory terms. If, on the other hand, one considers the pool of motoneurones generating the orienting response from activation of some part of the map, precision or registration takes on a new meaning. This has been most clearly pointed out by McIlwain[10,11] who has formulated some rules for representations within the superior colliculus. A very precise orienting response may be driven by a rather diffuse activation of output neurones within the motor map since the precision depends upon coordinated activity in a very large pool of different motoneurones. McIlwain has shown how, as the final motor output is approached, the size of the 'point image' activated within the array by a stimulus increases to include a significant part of the whole map.

The point image can be thought of as the spatial distribution of neurones which are activated within a map by a punctate stimulus in the appropriate sensory space. The size of the point image in the map is determined both by the receptive-field size of individual neurones and by the degree to which receptive fields of neighbouring neurones overlap. A remarkable feature of a number of visual maps, first noticed by McIlwain, is that the mapping and receptive fields are related in such a way that the point image at a given map location has the same size as a receptive field at that location when the receptive field is projected onto the map. Accordingly, the very large receptive fields encountered in the deeper layers of the superior colliculus will be associated with large pools of output neurones, to which increasing attention is now being paid by recording from behaving monkeys. Thus, the large and apparently imprecise auditory receptive fields of the neurones in monkey superior colliculus[2] belie the precision with which the monkey can fixate an auditory 'target'. Precision within a motor map is more a matter of defining the boundaries of the motoneurone pool than of limiting the stimulus to a particular location, so one must take care to establish what the map is designed to represent.

Motor considerations can also help one to understand why the monkey's auditory map should move coordinately with the eyes, instead of having the head and ears as a fixed reference frame as one might expect of a map generated from head and ear cues. Apparently, what is being mapped onto the superior colliculus is the error between the current sound source direction and the current visual axis. Since the superior colliculus of the monkey has a motor map of saccadic eye movement vectors, the combination of the two maps would enable eye movements to be accurately programmed to fixate an invisible sound source. We can thus think of a map in 'motor-error space'.

A requirement for the motor-error schema is that a map has available a neural representation of current eye position. Such a representation also forms an important part of most successful models

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of saccade-generation, the most notable of which (Robinson's bang-bang model) uses the degree of mismatch between a current-eye-position signal and an intended-eye-position signal to provide the drive for saccadic burst generators[12]. Just where the eye-position signals originate and how they are integrated with the map of auditory space remain difficult questions for the future, particularly in view of the current lack of information about how the auditory space map is generated in the monkey's midbrain. Future work on the midbrain maps therefore promises to illuminate a variety of important issues concerning the sensory–motor interface. □

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Zoology

Back from the brink of extinction

from Jared M. Diamond

A RECURRING nightmare for conservation biologists is the discovery that the few surviving individuals of an endangered species are all of the same sex. In 1976 this nightmare came close to reality for the New Zealand Wildlife Service, with the discovery that of seven remaining Chatham Island Black Robins only two were females. This precipitated a drastic rescue programme, involving capture of the entire robin population, its transfer to another island and the fostering of eggs in the nests of two other species of song bird. As a result, for the first time in nearly a century, robins now occupy two islands[1]. The significance of the rescue programme, however, goes far beyond the fate of one obscure species of bird because without such sophisticated management techniques a growing number of species would be doomed and yet the techniques arouse passionate opposition.

Why was the Black Robin (*Petroica traversi*) on the verge of extinction in 1976? By the 1870s the cats, rats and forest-clearing activities of European settlers had eliminated the robin from the larger of the Chatham Islands, 800 km east of New Zealand, restricting it to Mangere and Little Mangere. After cats were introduced to Mangere in the 1890s they proceeded to destroy its robins and 11 other bird species, leaving all of the world's Black Robins in 4 hectares of scrub on the windswept top of Little Mangere. By 1973 there were 17 adults and 1 juvenile[2,3].

On Little Mangere the robins' long-term prospects of survival seemed poor. Apart from the risks intrinsic in the island's tiny area, it was riddled with the nesting burrows of Sooty Shearwaters, its forest was dying and few young robins were surviving. After considering the options, the New Zealand Wildlife Service decided to plant 100,000 tree cuttings on Mangere Island itself (where the cats had died out), intending to transfer the robins there when the trees had become tall enough to provide suitable habitat. Meanwhile it began transfer experiments and detailed field studies with the Black Robin's closest relative, the New Zealand Robin (*Petroica australis*)[3].

Unfortunately, the continued deterioration of Little Mangere's patch of scrub, and the poor breeding success of robins there, made it impossible to wait. When the 1976 census revealed only two pairs and three bachelor males, making the Black Robin the world's rarest accurately censused bird, the Wildlife Service decided on a drastic risky measure: the immediate transfer of robins to the 4.2 hectares of existing scrub on Mangere.

Rough seas, bad weather and an almost fatal boat accident aborted the first two attempts[4]. But between 17 and 21 September 1976 both pairs and one bachelor were caught and transferred and at the end of the breeding season, in March 1977, the two remaining males joined them on Mangere[5] so that the whole population of the Black Robin was on the one island.

Although the birds immediately began to breed on Mangere, recovery of the Black Robin population was slow because only two eggs are laid, and at best only one of those is likely to yield a fledged young. However, like many bird species, Black Robins will lay a second or even a third clutch if the previous clutch fails. So, in 1980 the first clutches of the two surviving pairs were transferred to nearby nests of another small insectivorous bird species, the Chatham Island Warbler. The robins relaid, and four robin chicks were fledged that year — three of them by warblers. But the warblers were incapable of caring for the growing robin chicks for more than ten days, after which they had to be fostered back to adult robins. So, in 1981 the first clutches were transported to nearby South East Island for foster-rearing by the Tomtit (*Petroica macrocephala*). These proved ideal foster parents and raised three of the five robin chicks produced that year[1].

To reduce the risk of inbreeding or of extinction by a local disaster on Mangere, where the robin habitat is at the base of an unstable cliff, two pairs of adult robins were transferred to South East Island in 1983. Despite unusually bad weather leading to the deaths of four robins in 1982 and 1983, the species entered the 1983–84 breeding season with three breeding pairs.

These interventions obviously involved big risks, both to the Black Robin and to the New Zealand Wildlife Service itself. Even before the near-fatal boat accident in 1976, the Service was aware that public opinion would tolerate the loss of a ranger during the transfers, but not of either of the two surviving female robins[2]. The courage and experience required to move an entire population of an endangered species grew out of need. Faced with destructive introduced mammals on the New Zealand mainland, inadequate budgets and the responsibility for an unusually high proportion of endangered or vulnerable endemic species, the New Zealand Wildlife Service has developed management techniques based on detailed studies of the ecology and sociobiology of endangered species. Foremost among them has been the introduction of threatened taxa to offshore islands from which introduced mammals are absent or have been eradicated. Such taxa as the South Island Saddleback, Buff Weka and Little Spotted Kiwi would be extinct today were it not for such introductions.

Nevertheless, in New Zealand as elsewhere, the philosophy of drastic intervention to save endangered species elicits opposition as well as support, even amon biologists. The debate over whether t transfer some Black Robins from Mangere to South East Island is not unlike the debate in the United States over the captive breeding programme for the California Condor. Sometimes opposition is based on doubts about the particular form of intervention proposed, the risk of individuals being killed and the possibility that a programme will draw off too much money from other conservation programmes. Partly, too, the opposition stems from revulsion at intense artificial manipulation of wild populations, even in the hope of saving them: should not the last condors be allowed to die in dignity in the wild, rather than as captives in a zoo? Breeding of Black Robins will have to be managed for a while to avoid effects of inbreeding, and perhaps to avoid imprinting on foster-parents. Does this not blur the distinction between maintenance in the wild and in a zoo, and thereby virtually convert Mangere and South East Island into outdoor zoos?

Many people, however, feel that managed robins are better than no robins and that humans should devote ingenuity and massive intervention to saving species as well as to exterminating them. Without intervention in 1976 the Black Robin would soon have met the fate of the Dusky Seaside Sparrow. For this doomed bird of Florida marshes, the conservationists' nightmare has come true: the few surviving individuals are all males. □

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Protein crystallography

Fifty years of pepsin crystals

from Guy Dodson and Cyrus Chothia

In the middle of April 1934, J.D. Bernal and Dorothy Crowfoot (now Hodgkin) obtained the first successful X-ray photographs of a globular protein. They were obtained in Cambridge from crystals of pepsin grown by John Philpot in Uppsala. The photographs, discussed in a letter to *Nature* (**133**, 794; 1934) published half a century ago, almost to the day, were the start of one of the great achievements of modern biology.

On 13 April 1984 a meeting was held in Cambridge to commemorate the fiftieth anniversary of the experiment. Lectures were given by Dorothy Hodgkin, Max Perutz and Aaron Klug, all of whom worked at different times in Bernal's laboratory. The occasion brought together all those still living who were directly involved in the pepsin experiment; sadly, Peter Wooster, a long-established figure in Bernal's laboratory and remembered warmly by many, died on the night preceding the meeting. Several crystallographers who had contributed to the field, notably Arthur Wilson (intensity statistics) and Henry Lipson (Fourier calculation methods), were also present.

Part of the meeting was devoted to a recreation of the events that led to the pepsin crystal's coming to Bernal's laboratory at Cambridge and to the crystallographic examination of pepsin and other protein crystals (including viruses) that subsequently took place. The historical accounts were filled out, sometimes straightened out, particularly by John Philpot on the background of the crystallization of pepsin (in explaining why he went to Uppsala to grow the crystals he distinguished nicely between his real reason for going there — a young lady — and his excuse — to work on the ultracentrifuge) and by Bill Pirie on early virus experiments. The history of pepsin was completed by a comment from Tom Blundell on the structure of the pepsin molecule inferred from homologous enzymes.

The impressions that emerged of Bernal's laboratory were of great activity and many abilities, and of Bernal's omnivorous scientific interests. The laboratory already had a famous reputation, particularly for the work on sterols, and it was this, or Dorothy Crowfoot's reputation, that apparently prompted Glen Millikan, a peripatetic scientist, to bring John Philpot's crystals to Cambridge.

When the crystals arrived, Bernal immediately examined them in a polarizing microscope (Dorothy Crowfoot was away with what proved to be an incipient rheumatic attack). He found them to be beautifully formed, showing sharp birefringence while in the tube they had been grown in. The crystals failed to diffract after removal from the test tube and drying but, crucially, Bernal noticed that their birefringence had also disappeared. Thus he realized the need to keep crystals wet. Bernal's solution was to put the crystals in capillaries (made possible because of Helen Megaw's studies on ice crystals). The subsequent investigation of the crystal's diffraction characteristics, cell dimensions and space group was carried out by Bernal and Dorothy Crowfoot. It led to their recognition "that the arrangement of atoms inside the protein molecule is of a perfectly definite kind" and to remarkably accurate ideas about the molecules' molecular weight and shape.

The descriptions of the subsequent research into protein crystals included the details of how the studies on insulin, haemoglobin and the viruses began. The immense difficulties in solving these structures were clearly appreciated at the time and the early investigations were devoted to measuring X-ray intensities, to calculating the Patterson function (also celebrating its fiftieth anniversary this year) and to characterizing new protein crystals. The influence of Bernal and Bragg was clearly crucial. A typical contribution was Bernal's letter to Dorothy Crowfoot suggesting that the isomorphous replacement of Zn by Cd in the rhombohedral insulin crystal could give phase information. Bragg's role, more concentrated at first on haemoglobin and later on myoglobin, was quieter but his steady sympathy, persistence and deep crystallographic insight helped the early research through disappointing years. Perutz recalled Bragg's grasp of crystallographic principles and illustrated it by the studies on how the shrinkage of protein crystal cells could be used to define centric phases.

In his review of virus research and the structure of water, both deep interests of Bernal, Klug emphasized the importance of flexible thinking and how Bernal's aphorism, 'crystallographers must learn not to be crystallographers', applied over and over again. This was as true in Pauling's production of the α-helix structure and in Bernal's approach to the water problem as it is now in the study of the behaviour of protein structure which is increasingly found to link well defined domains to flexible and variable segments. Thus, the linking of order and disorder to achieve assembly and function (RNA binding) in the tobacco mosaic virus is revealed by crystallographic analysis. A report by Steven Harrison (Harvard University) of the 2.8 Å resolution crystal structure of tomato bushy stunt virus nicely completed studies begun by Bernal and Fankuchen. This virus also demonstrates beautifully the order–disorder phenomenon, which Harrison showed is related to the packing and assembly of the virus subunits.

There are many conclusions from a day like this. First, there are the remarkably happy collaborative traditions in protein crystallography, established from the earliest days, that make it such fun to work in the field. Second, the techniques, essentially physics, have put the description of protein and biomolecular structure into the hands of crystallographers. Finally, the importance of crystals and other ordered systems as a route to the description of biochemical and biological structure remains paramount. Bernal saw these descriptions as the first stage — their analysis should lead to the principles which govern the assembly and integration of molecules into higher organizations. Already, as Richard Henderson emphasized, crystals of very large molecular assemblies such as the nucleosome have been solved. Thus, as long as there are crystals, the future of the field is assured and more than justifies the great excitement produced by those first X-ray photographs of pepsin. □

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Dorothy Hodgkin, John Philpot and Helen Megaw at the meeting. (Photograph by Claudio Villa.)

Crystallography

Defects in reduced oxides

from C.J. Humphreys

CRYSTALS are like people: it is the defects in them which tend to make them interesting. In recent years, solid-state physicists and chemists have become increasingly aware that real crystals are usually not perfectly periodic arrays of atoms. Instead they contain a variety of isolated defects, which are important because they can strongly influence the physical and chemical properties of the crystals. An article on page 319 of this issue, by Bursill and Smith, reports the use of high-resolution electron microscopy to provide new information on the isolated defects which form when oxide crystals are reduced. This is the latest development in understanding reduced oxides, which have long puzzled chemists and physicists.

The basic question is the following: when oxide crystals are reduced (for example, $MoO_3 \rightarrow MoO_{3-x}$), are the resulting oxygen vacancies in the structure distributed homogeneously or are there isolated extended defects, and if so how do they form? Until 1950 it was believed that the oxygen vacancies were distributed homogeneously. Then A. Magnéli (*Ark. Kemi* **1**, 513; 1950) observed extra diffraction spots in X-ray diffraction patterns from reduced oxides which he correctly attributed to an ordered array of defects called crystallographic shear planes (CSPs). Five years later A. D. Wadsley postulated the existence of isolated CSPs (*Rev. pure appl. Chem.* **5**, 165; 1955). But the then available techniques of X-ray and neutron diffraction gave diffraction patterns which yielded atomic positions statistically averaged over a relatively large diffracting volume (typically 1 mm^3) of the crystal and so were incapable of detecting individual crystal defects. It took another fourteen years before Wadsley's predictions were confirmed by the observation of isolated CSPs by electron microscopy (Bursill, L.A. & Hyde, B.G. *Phil. Mag.* **20**, 657; 1969).

We can think of CSP formation as follows. If it is energetically favourable for them to do so, oxygen vacancies will cluster together on a crystal plane rather than exist as isolated vacancies. The crystal on each side of this cluster of vacancies will then collapse into the missing layer of oxygen atoms and also shear sideways to reform chemical bonds. The defect so formed is a CSP. In MoO_3 the local chemical composition at a CSP is MoO_2. Hence a reduced crystal of overall composition MoO_{3-x} can be thought of as a matrix of MoO_3 plus the appropriate number of CSPs of composition MoO_2. We thus have a beautifully simple concept of how oxygen vacancies are accommodated in reduced oxides. If there are a number of CSPs in a crystal they will form an ordered array at a sufficiently high temperature, as Magnéli originally observed.

The picture I have just given is a simplified one. In particular, for a very small degree of reduction (corresponding to $x<0.005$) CSPs are not usually observed. Are the oxygen vacancies at these low concentrations uniformly distributed or are they concentrated into isolated small defects, and how do the CSPs form and grow? These are the questions that Bursill and Smith can address in their paper because of the availability of the latest generation of very high-resolution electron microscopes, with a resolution of less than 0.2 nm. They show, for the first time, that there are small localized defects which precede the CSPs and that these defects may be characterized through careful analysis of very high-resolution electron micrographs. Thus we gain new insight into the earliest stages of structural changes that accompany reduction. This work is not only important for chemists and physicists studying oxides but also illustrates the power of high-resolution electron microscopy for studying, at the atomic level, the structure of defects in a wide range of materials. □

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Immunology

More on the T-cell receptor

from Philippa Marrack

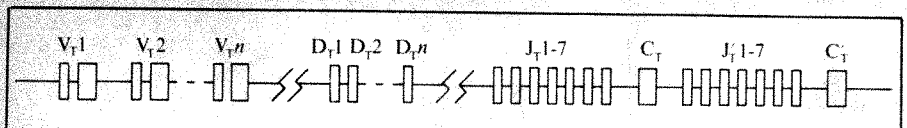
NOT surprisingly, one of the main subjects of interest at a recent symposium* on 'Regulation of the immune system' was the receptor on T cells which allows them to respond clonally to antigen in association with products of the major histocompatibility complex (MHC). A year or two ago, when the molecules responsible for this feat were identified on the surfaces of T-cell clones, using clone-specific antibodies, they were shown to be disulphide-linked proteins made up of two different chains each of molecular weight 40,000–50,000 and containing variable and constant peptides. The T-cell receptor was therefore recognized to be strikingly similar in overall design to immunoglobulins. Recent papers in *Nature* (**308**, 145 & 149) reported the isolation of cDNA clones which probably encode one of the two polypeptide chains.

Since nearly all the protagonists involved in these discoveries were at the meeting, immunologists were given an opportunity to be brought up to date on all aspects of this work. Steven Hedrick (University of California, San Diego) and Tak Mak (Ontario Cancer Center) reviewed the data on the cDNA clones. The mRNAs from these clones are T-cell specific, come from genes which rearrange between the germ line and mature T cell, and are similar but not identical to immunoglobulins in nucleotide sequence. Moreover, the proteins they encode are very immunoglobulin-like in sequence and, in particular, include cysteines which could mark the domain-like structures so characteristic of this type of molecule. Hedrick also pointed out that the cysteine near the carboxy-terminal end of the protein is in a position analogous to the cysteine on immunoglobulin light chains. Since the latter is involved in bridging light chains to heavy chains in most immunoglobulins, the former may have a similar function in the T-cell receptor, being used to bridge the two chains of the heterodimer.

Mark Davis and Nicholas Gascoigne (Stanford University) described the structure of mouse genomic clones encoding these sequences; their data are published on page 322 of this issue. Again there are striking similarities with immunoglobulin, since several segments of DNA, which occupy separate loci in the germ-line genome but are brought together by rearrangement in T cells, contribute to T-cell receptor cDNA (see the figure). One segment contains a cluster of variable-region (V_T) genes, mapping an undetermined distance from a proposed segment containing several diversity-region (D_T) genes (DNA encoding this segment has not yet been found). A third segment contains a cluster of seven joining-region (J_T) genes and a constant-region (C_T) gene. The last contains four exons, of which three were expected: the exons for the constant-region immunoglobulin-like domain, which is extracellular, a transmembrane sequence, and a cytoplasmic domain. But the fourth, encoding four to six amino acids between the extracellular domain and the transmembrane sequence, came as a surprise. This extra domain has some sequence homology with the hinge region of immunoglobulins, the part thought to confer flexibility on antibodies. A lack of prolines in the hinge region of the T-cell receptor suggests it might not be quite so flexible. The cysteine equivalent to that thought to be involved in interchain disulphide bonds in antibody molecules is found in this exon.

Davis and his co-workers have found

*The Ortho-UCLA symposium on 'Regulation of the immune system' was held in Park City, Utah, 18–25 March.



Arrangement of the genes for the separate domains of one chain, probably the β chain, of the T-cell receptor. Bars represent genes. The D_T region is provisional.

two very similar C_T genes, close together on the same genomic clone. Each seems to have its own cluster of J_T genes; there is evidence that each V_T gene can feed off either cluster of J_Ts. If so, this arrangement would be similar to that of the immunoglobulin light chain, λ. These results were confirmed by Mitch Kronenberg (Caltech). The human gene structure seems to be similar since Mak has also found two closely arranged constant-region genes. In mouse the genes map to the proximal region of chromosome 6 (Davis and Kronenberg).

There is some evidence that the T-cell repertoire, at least reflected by use of these genes, is limited. For example, there is no evidence for somatic variation, although so far only a limited number of sequences have been looked at. Davis mentioned that the V_T-region sequence found in a T-cell hybridoma specific for pigeon cytochrome *c* in association with $I\text{-}A^k$ is identical to its germ-line counterpart. Perhaps less surprisingly, Hedrick showed that three independent T-cell hybridomas with the same specificity for cytochrome *c* and I-A seem to use the same V_T region, at least for this chain, since all show the same size rearrangement of the gene.

Which of the two polypeptides, unimaginatively called α and β, of the T-cell receptor are coded for by the genes detected by the cDNA probes? Ellis Reinherz (Harvard University) and his collaborators have shown that the amino-terminal sequence of the variable region described by Mak is almost identical to the amino-terminal amino acid sequence they have obtained for the β chain.

With every indication that the arrangement and rearrangements of T-cell receptor genes have been, or soon will be, worked out, interest turned to their expression. All investigators agreed that the genes are rearranged and transcribed only in T cells although not, perhaps, in suppressor T cells. The high level of mRNA produced from the genes in thymocytes is of particular interest because the thymus is the organ in which the ability of T cells to recognize antigen in the context of self-MHC is thought to be selected. Presumably this selection depends on the expression of receptor proteins on thymocyte surfaces. Some doubt that high levels of mRNA necessarily result in high levels of cell-surface receptor was raised by John Kappler (National Jewish Hospital, Denver), who described a monoclonal antibody which binds to the receptors on about 20 per cent of peripheral T cells in most mouse strains. Each of these cells has $3\text{–}4 \times 10^4$ receptors on its surface whereas the number of receptors on immature thymocytes ranges from zero to about the same as on the peripheral T cells. Those without any receptors may not yet have generated the ability to synthesize mRNA for both chains of the receptor, or may have lost this ability because of mutation or aberrant rearrangement.

Since cytotoxic T cells usually recognize antigen in association with class I products of the MHC, whereas helper T cells usually recognize antigen in association with class II MHC products, some difference between the receptors on the two types of cells has long been expected. Although no differences were identified with the cDNA probes or with Kappler's antibody, a first inkling of their existence was suggested by J.P. Allison (University of Texas Cancer Center, Smithville) who showed that antibodies produced in rabbits against the purified receptor from a mouse T-cell leukaemia preferentially react with a subset of receptors on L3T4-bearing mouse T cells, the cells thought to be mainly class II-restricted helper T cells.

Some years ago Jerne suggested that an immunological network controls the specificities of lymphocytes in any given animal since each new variable-region sequence is, in a sense, an antigen new to the animal and so potentially recognizable by other variable regions — of antibodies or on T cells. Hence the suggestion that there might be a subset of T cells which recognizes the T-cell receptor on other T cells and does so by means of a receptor that bears some resemblance in structure to the MHC antigens recognized by the T-cell receptor. Sim and Augustin (Denver) supported that suggestion with evidence that a T-cell hybridoma, prepared from mice immunized with an anti-I-A antibody, responds to that antibody in the absence of any other stimulus, and that the antibody can precipitate from the hybridoma a structure similar to other antigen-specific MHC-restricted T-cell receptors.

The idea that T cells might bind anti-MHC antibodies, in particular those directed against class II-like molecules, not because they bear products of the MHC itself but because they bear receptors which resemble MHC products (internal images), was used at several points in the meeting to explain the still mysterious existence of antigens on T cells which seem to map genetically in the *I* region, especially the *I–J* region, of the MHC. But why, then, are *I*-region-related messages not expressed in T cells (Kronenberg)? Colleen Hayes (University of Wisconsin) and her colleagues have recently published data which suggest that the determinants found on T cells known as I–J are controlled in the mouse by genetic loci mapping both in the MHC, on chromosome 17 and on chromosome 4, suggesting some sort of complementation (*Science* **223**, 559; 1984). Internal-image devotees now theorize that I–J, and other structures on T cells which react with anti-MHC antibodies, may be T-cell receptors which mimic the MHC but which are encoded by genes not mapping at this locus. This may be true for MHC-restricted helper and cytotoxic T cells, as exemplified by the Sim and Augustin experiment, and for free-antigen-binding suppressor T cells, which apparently have a different type of receptor. □

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100 years ago

NOTES

M. PASTEUR read to the Academy of Sciences on Monday an account of his experiments on rabies. He maintains that he has twenty dogs which he has rendered insusceptible to the disease, and which, with twenty ordinary dogs, he is prepared to have bitten by a number of dogs in a rabid state. A Commission has been appointed by the French Government to test M. Pasteur's conclusions, the immense importance of which, if established, must be evident to every one. Eminent physiologists maintain, however, that M. Pasteur is far from having proved his position, and that it would be rash to give any positive opinion upon the subject until the experiment which he suggests has been made. We await the full report of M. Pasteur's paper before saying more upon it.

From *Nature* **30**, 22 & 29 May 1884.

A REMARKABLY BRILLIANT METEOR

TO-NIGHT, about 10.45 p.m., I was "stepping westward," about half a mile east of my house. Suddenly the ground before me was lighted up with moontide splendour by a luminary that was above me and behind me. Looking back I saw a meteor a good deal east of the Grat Bear, and nearly as high in the sky. It was about as big as Venus, and of the same hue. It was speeding from north to south with a slight descent. Its course very soon came to an end. It left behind it a streak of duller lustre: this phosphorus-like trail vanished almost at once. The career of this meteor while that body was visible here, lasted little, if at all, longer than a minute, but its light was remarkably brilliant.

DARK TRANSIT OF SATELLITE

ON May 18, at 8 h., on observing Jupiter with my 10-inch reflector, p. 252, I saw three very dark spots. These I took to be the shadows of satellites, and on reference found that the shadows of Satellites I. and II. were really upon the planet; also Satellite I. itself. I believe the very dark appearance of the satellite on this occasion to have been somewhat exceptional; for through I have observed a considerable number of its transits, I never saw it nearly so dark before.

Molecular biology

DNA bending and kinking

from Jonathan Widom

To pack double-stranded DNA into the protein shell of viruses such as phage λ, or to wrap it around the protein cores of the nucleosomes that characterize the chromatin of higher organisms, requires the DNA to be tightly bent or kinked at many sites. In the simplest case, DNA may have only a passive role in its packaging. In other cases, sequences within the DNA may specify the bending or kinking, or bends or kinks could be induced by the binding of proteins. Experiments described in a recent issue of *Nature*[1] and on page 327 of this issue[2] provide evidence of both of the active mechanisms of bending or kinking.

To understand the degree of bending required of DNA packaged in viruses and in chromatin, one must have a quantitative measure of the stiffness of isolated DNA. This has been obtained by hydrodynamic methods[3], and extended to tight bending (~ 240 base pair circles) by Shore *et al.* using an enzymatic ring-closure method[4]. The persistence length of random-sequence DNA is found to be ~500 Å, or ~150 base pairs (bp)[3]. DNA must be tightly bent or kinked when packaged in phage λ because the hollow protein capsid has a diameter of only 600 Å (ref.5). On a nucleosome, DNA is bent through 360° in the space of ~ 80 bp (ref.6), only half of the persistence length of DNA in solution.

Adding to the interest in what determines bends or kinks in DNA is the possibility that altered DNA conformation may serve as an important signal in the sequence-specific binding of proteins. Thus in 1975, Crick and Klug[7] pointed out that several base pairs at a kink may be particularly accessible to proteins and, on the basis of model-building studies, several groups have proposed non-B-form conformations for DNA bound to proteins (for example, refs 8 and 9). Although the structures of three sequence-specific DNA-binding proteins (cro[10], cI[11], CAP[9]) have been solved by X-ray diffraction, the proteins were not bound to DNA, so only by model-building could their mode of binding be tentatively predicted. That is the reason for the special importance of the two new papers[1,2] which clearly show striking departures from linear B-DNA in both isolated DNA and in DNA–protein complexes.

Wu and Crothers report an elegant experiment which indicates that a particular DNA sequence may cause the DNA molecule to bend[1]. The DNA they have worked with is a restriction fragment from the kinetoplast of a trypanosome. The kinetoplast contains a large number of highly-interlocked, very small (~ 800 bp) DNA circles of unknown function. Marini *et al.* had reported anomalous electrophoretic and hydrodynamic behaviour for one particular restriction fragment, which led them to postulate that the fragment may be bent[12]. To test that suggestion, Wu and Crothers devised an elegant technique (circular permutation) of generating from the anomalous restriction fragment a series of variants differing only in the position of the suggested bending locus relative to the ends of the fragment. When these essentially identical restriction fragments were run on non-denaturing acrylamide gels, a striking range of mobilities was observed, consistent with the proposal that the original fragment was bent at a specific site. Theories of gel electrophoresis[13] predict (for a given molecular mass) a dependence of mobility on h^2, the mean-squared end-to-end distance of the molecule. If a sequence at the centre of a restriction fragment causes that molecule to bend, it will have a reduced h^2 and hence should migrate more slowly in gels. If that same restriction fragment is circularly permuted to place the site of bending at the very end of the molecule, h^2 should be restored to its expected value, so the fragment should migrate more rapidly. By extrapolating their mobility data, Wu and Crothers are able to pinpoint the DNA sequence that causes the bend. In the restriction fragment from the kinetoplast minicircle (K-DNA), they find a very striking sequence at the centre of the bend: the sequence $C(A)_{5\text{-}6}T$ is repeated four times, separated by three or four other bases so that the sequences are spaced ~ 10 bp apart, in phase with the twist of the DNA helix.

Theories of gel electrophoresis are not sufficiently developed to allow one to be certain of the reason for the anomalous mobility of K-DNA. An alternative explanation, not considered by Wu and Crothers, is that rather than conferring a permanent bend with definite geometry, the sequence $C(A)_{5\text{-}6}T$ could simply confer increased flexibility, and that increased flexibility results in slower migration in gels. Hogan *et al.*[14] recently reported enhanced flexibility for the sequence poly(dA)·poly(dT), and Charney and colleagues (personal communication) have found a greatly enhanced flexibility for the sequence poly(dA-dT)·poly(dA-dT).

Although it is therefore reasonable to expect that the sequence found by Wu and Crothers will have an increased flexibility, two lines of reasoning suggest that increased flexibility alone cannot account for their results. First, Charney and colleagues find that, while having substantially enhanced flexibility, poly(dA-dT)·poly(dA-dT) does not behave anomalously on polyacrylamide gels. Second, increased flexibility would not account for the striking 5 and 10 bp periodicities of the K-DNA bend locus. Wu and Crothers suggest that runs of $dA_{5\text{-}6}$ may have an altered helical conformation, such as the heteronomous DNA (H-form) postulated by Arnott and colleagues[15]. When a B-form to H-form junction is created, it is likely to involve a bend[15]. The two strands of H-form DNA are non-equivalent, so there will be a directionality to an H–B junction (with respect to the polar phosphate–sugar backbone), producing bends of opposite sense at H→B and B→H junctions. Because the runs of A are 5–6 bp long — half of the helical periodicity of DNA — bends of opposite sense at the entrance and exit of $A_{5\text{-}6}$ blocks will point in the same direction to produce a planar bend rather than a zigzag. There are four of these units in the K-DNA bending locus; because the units themselves repeat every ~ 10 bp, planar bends from individual units add in-phase to produce a large net planar bend. If these sequences promoted bending merely by virtue of their flexibility, the 5 bp periodicity, especially, would be unnecessary and not expected.

In a parallel series of experiments, Wu and Crothers showed that the mobility of circularly permuted variants of a restriction fragment from the *lac* control region of *Escherichia coli* does not vary with circular permutation, even when lac repressor tetramers were bound at the operator site. When dimers of CAP protein (which are much smaller than lac repressor tetramers) were added, however, there was a marked dependence of the mobility on the degree of circular permutation. Previous experiments have shown that both CAP and lac repressor remain correctly bound to the DNA during electrophoresis (see refs 1,16). Wu and Crothers conclude that the *lac* DNA fragment is itself unbent but that it bends in response to the binding of CAP protein. The same conclusion based on related experiments had previously been reached by Kolb *et al.*, who obtained fragments with the CAP-binding site at different locations relative to the fragment ends from many different control regions of *E. coli*[16]. By extrapolating their mobility data, Wu and Crothers map the CAP-induced bend to a point within the CAP-binding site that is several bases towards the promoter from the binding site centre of symmetry. This raises the possibility that CAP acts to promote transcription by stabilizing an altered DNA conformation which may be favoured by RNA polymerase.

Turning to the paper of Frederick *et al.*, on page 327, one is presented with the striking results of the first X-ray diffraction structure of a complex of protein and DNA[2]; the results bear directly on the issues of sequence-specific recognition and DNA packaging. Frederick *et al.* have studied the complex of the restriction enzyme *Eco*RI with DNA, formed in the absence of Mg^{2+} so that the enzyme did not hydrolyse the DNA. The DNA has the

sequence: TCGCGAATTCGCG
GCGCTTAAGCGCT

That sequence contains the recognition sequence for *Eco*RI, GAATTC, and is closely related to the dodecamer studied by Dickerson and colleagues[17]. Frederick *et al.* find that the protein-bound DNA has several unusual features, including three kinks. One kink (the 'neo-1' kink) is in the centre of the DNA molecule, on the dyad symmetry axis, and represents a torsional dislocation between two segments of B-form DNA. One can think of this kink as being generated in two steps: first, the DNA is unwound by 25° between the base pairs to either side of the kink, then the DNA is bent 12° into the minor groove. This is the mechanism of kinking postulated by Crick and Klug[7] in their kinked-helix model, but the present neo-1 kink differs from the Crick–Klug kink by restricting itself to a modest 12° bend, rather than taking advantage of the full 100° bend that model-building suggests is available. In the complex with *Eco*RI, the neo-1 kink is required to widen the major groove, facilitating access to the bases and allowing an extensive complementary interface with the protein dimer.

The other two kinks ('neo-2 kinks') differ from the neo-1 kink, but are identical to each other because they occupy equivalent positions about the complex's dyad axis. The neo-2 kinks separate the terminal (T)CGC blocks from the central GAA blocks and are produced in a different way from the neo-1 kink. They lack noticeable unwinding; a base-roll[17] into the minor groove produces a bend of 23°. While the central GAA blocks appear to be B-like, the terminal (T)CGC blocks appear to be A-like. Frederick *et al.* suggest that the neo-2 kink may arise from the joining of these two different DNA conformational blocks. It should be noted that in a previous model-building study, Selsing *et al.*[18] found that they could not build a stereochemically acceptable A–B junction of the sort proposed here. Selsing *et al.* found it necessary to have at least one intervening base pair separating orthodox A-and B-form regions. A better understanding of the neo-2 kink must await determination of the atomic positions of the base pairs involved.

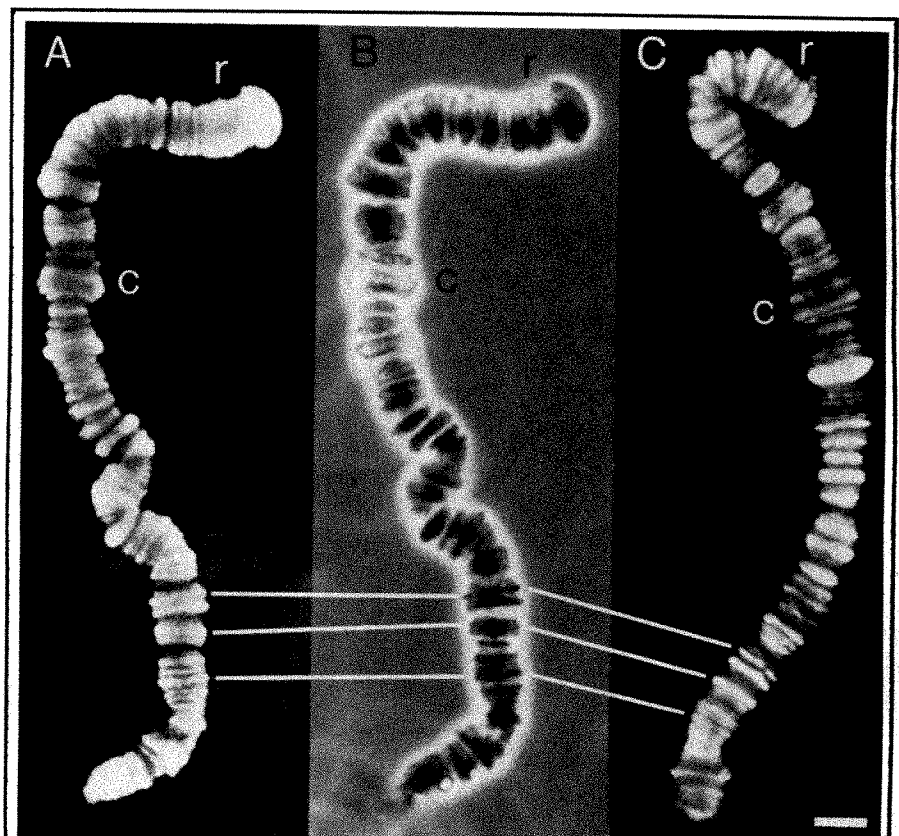
A final striking feature of the *Eco*RI–DNA complex concerns the effects of DNA sequences flanking the enzyme's recognition sequence, GAATTC. Different flanking sequences are known to influence the hydrolysis rate by an order of magnitude[19]. Surprisingly, therefore, Frederick *et al.* see no contacts between *Eco*RI and DNA bases outside the recognition sequence; only the backbone appears to be contacted. They conclude that the flanking sequences may affect enzyme activity by changing the conformational free energy of the neo-2 kink or of the GCC terminal blocks. An alternative possibility is that different flanking sequences may give rise to different local DNA backbone conformations, for which the enzyme may have different affinities.

One must be aware that *Eco*RI is a hydrolytic enzyme, whose function is to cut the DNA chain between the bases G and A in the sequence GAATTC. The site of cleavage is only 1 bp away from the neo-2 kink and 2 bp away from the neo-1 kink. Presumably the enzyme acts by stabilizing a transition state in which the G-A backbone has an increased lability to hydrolysis. It is feasible that either the neo-2 kink or the neo-1 kink may destabilize the G-A backbone bond, and indeed this may turn out to be the *raison d'être* of the neo-2 kink. This does not detract from the important conclusion that for both CAP and *Eco*RI, and probably many other proteins, dramatically altered DNA structures will be important features in the DNA–protein complexes.

Taken together, the new studies of K-DNA and of CAP–DNA and *Eco*RI–DNA complexes show that the structure and function of a DNA molecule can be affected in important and unexpected ways both by the DNA sequence itself and by the interaction of specific proteins with the DNA. These results suggest that altered DNA conformations are likely to play an important part in DNA packaging and in the sequence-specific recognition of DNA by proteins. □

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Z-DNA: the acid test

Acid treatment of polytene chromosomes of *Drosophila* before their exposure to an antibody against Z-DNA resulted in these striking immunofluorescent micrographs. A phase-contrast image of an isolated chromosome (B) allows its bands to be clearly identified and makes obvious that Z-DNA is associated with them, both in an acid-treated isolated chromosome (A) and in a 'squash preparation' of a chromosome prepared after acid fixation of the salivary gland (C). In less acid conditions, far less Z-DNA is apparent and it is confined to the periphery of the bands; acid treatment, by stripping proteins from the DNA and by protonating its bases, may both expose masked Z-DNA and induce the transition of B-DNA to Z-DNA. The lines connect bands, 'c' denotes centromere, 'r' the right-hand end of the chromosomes and the bar represents 10 μm. From Robert-Nicoud, M., *et al. EMBO J.* 3, 721 (1984).

Mathematics

Beyond reasonable doubt?

from Ian Stewart

WHEREAS the mathematical discipline of Number Theory deals with properties of whole numbers, that of Analysis is concerned with the continuous properties of real and complex numbers. Despite this difference, Bernhard Riemann, in 1859, discovered profound connections between the two, leading to what is now known as Analytic Number Theory. Riemann developed a theory of the zeta function, defined for real $s > 1$ by

$$\zeta(s) = 1^{-s} + 2^{-s} + 3^{-s} + \ldots$$

and for complex s by analytical continuation, and showed how it could be used to study the distribution of prime numbers (*Monatsber. Akad. Berlin*, 671; 1859). In particular, the number of primes in a given interval could be estimated provided enough was known about the zeros of the zeta function: values s such that $\zeta(s) = 0$. There are some relatively obvious zeros at $s = -2, -4, -6, \ldots$. Riemann conjectured that all the remaining zeros lie on the complex line $s = \frac{1}{2} + iy$ where y is real. This, the Riemann Hypothesis, if true, has extensive repercussions — not all within Number Theory. It remains an intriguing and important unsolved problem.

Computer calculations, based on various known features of the zeta function, have shown that Riemann's hypothesis holds good for the first 320 million zeros (Edwards, H.M. *Riemann's Zeta Function*, Academic, New York; 1974). This would appear to be strong evidence in favour of the hypothesis — but is it? An analogous question is illuminating. In 1885 T.J. Stieltjes came up with a different conjecture which would, if true, imply the Riemann Hypothesis (*C. r. hebd. Seanc. Acad. Sci., Paris* **101**, 368). It was proposed again by F. Mertens in 1897, after whom it is now named (*Sber. preuss. Akad. Wiss.* **106**, 761). It can be stated as follows. For any integer n, let $M(n)$ be the difference between the number of integers less than n that are products of an even number of distinct primes and the number that are products of an odd number of primes. The Mertens Conjecture is that $M(n) < \sqrt{n}$ for $n > 1$. For example when $\sqrt{n} = 16$ there are five numbers in the 'even' case (1,6,10,14,15) and six 'odd' (2,3,5,7,11,13), so $M(16) = 1$ which is certainly less than 4. The Mertens Conjecture has been verified by computer calculations for the first 10 billion values of n.

Despite the apparently overwhelming evidence in its favour, it has recently been discovered that the Mertens Conjecture is false. Andrew Odlyzko (Bell Laboratories) and Herman te Riele (Centre for Mathematics and Computer Science, Amsterdam) have shown in unpublished work, based upon fast computer techniques, that there exist infinitely many values of n for which the conjecture fails. The first 'bad' n is no larger than 10 to the power 10 to the power 70 (or thereabouts), an order of magnitude totally inaccessible to direct computation. It is possible that an explicit calculation of the actual 'bad' values would not be an improvement on the demonstration that they must exist.

The Riemann Hypothesis is unaffected by this result: it is still unsolved. Experts have long felt that to prove it by way of the Mertens Conjecture would be 'too easy', and their suspicions are now vindicated. Meanwhile, if the first 10 billion numbers are conspiring to mislead us about the Mertens Conjecture, what faith can we place on a mere 320 million in favour of the Riemann Hypothesis? □

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Astronomy

IRAS circular 11

THE sources in this circular all have a firm detection at 60 μm, are at least 20° away from the galactic plane and have continua representative of 'warm' galaxies. Each search box has been inspected on SERC/ESO Schmidt survey plates or on National Geographic/Palomar Schmidt plates, and contains an obvious candidate, in most cases a galaxy. Selection of sources and the optical search were done by G.K. Miley and M.H.K. de Grijp, Sterrewacht Leiden, The Netherlands. Source names derived as before; see *Nature* **309**, 480; 1983. Position is given at equinox 1950.0. Measurements were made between epochs 1983.1 and 1983.9. In the table, a m denotes arc min.

Also now available, but not to be published in *Nature* is IRAS circular 10 which lists 179 sources selected from the IRAS minisurvey of an area of approximately 300 square degrees between 9 and 16 February 1983 (see Rowan-Robinson *et al. Astrophys. J. Lett.* **278**, L7; 1984). The sources satisfied the criteria of being more than 20° away from the galactic plane, reproducible on successive scans and with a signal-to-noise ratio of greater than 9:1 in at least one of the wavelength bands.

Source IRAS	RA h min s	Dec deg a m	Flux density (Jy) 12 μm	25 μm	60 μm	100 μm
0425−072P11	04 25 22.2	−07 15	<0.4	0.4	0.9	1.3
0425−046P11	04 25 57.1	−04 40	<0.2	1.6	4.5	4.3
0428−097P11	04 28 11.0	−09 44	<0.3	0.4	0.7	<1.4
0432−143P11	04 32 32.5	−14 19	1.2	3.8	7.9	11.3
0438−084P11	04 38 28.8	−08 28	0.5	1.8	3.4	2.8
0450−032P11	04 50 14.1	−03 17	<0.6	0.5	1.0	1.5
0450−184P11	04 50 40.8	−18 26	<0.2	<0.5	0.9	2.5
0509−024P11	05 09 03.8	−02 26	0.3	1.2	2.0	<1.5
0513−235P11	05 13 44.2	−23 31	<0.2	<0.5	0.9	3.1
0521−122P11	05 21 47.0	−12 12	<0.2	0.4	0.6	<2.8
0531−206P11	05 31 57.0	−20 36	<0.2	<0.4	1.2	2.0
0556−348P11	05 56 31.9	−34 53	<0.7	<0.3	0.5	<1.3
0611−326P11	06 11 30.1	−32 40	<0.2	0.4	0.9	1.5
0815+035P11	08 15 18.0	+03 31	<0.4	<0.3	0.7	<1.7
0818+033P11	08 18 49.8	+03 19	<0.2	<0.4	0.8	2.0
1036−190P11	10 36 39.5	−19 04	<0.4	<0.3	0.6	<1.4
1051−273P11	10 51 09.1	−27 22	<0.2	0.4	1.0	<1.2
1105−115P11	11 05 48.9	−11 31	<0.2	0.4	0.8	<1.4
1119+045P11	11 19 55.6	+04 31	<0.7	0.5	0.9	2.7
1121−281P11	11 21 33.3	−28 06	<0.4	0.4	0.7	<0.8
1246−111P11	12 46 53.3	−11 07	<0.2	0.8	1.7	2.1
1249−131P11	12 49 35.1	−13 08	<0.2	<0.5	1.3	2.8
1304−234P11	13 04 23.5	−23 24	0.4	1.3	2.6	4.1
1305−241P11	13 05 59.1	−24 07	<0.2	0.7	1.6	2.1
1315−098P11	13 15 31.4	−09 49	<0.2	<0.5	0.9	<1.6
1316−242P11	13 16 49.3	−24 13	<0.2	<0.4	0.9	<2.1
1319−164P11	13 19 42.3	−16 27	0.8	2.9	6.3	7.1
1320−342P11	13 20 44.8	−34 15	<0.4	<0.3	0.6	<1.7
1329+022P11	13 29 19.7	+02 16	<0.2	<0.6	1.1	1.2
1331−301P11	13 31 28.9	−30 07	<0.2	<0.3	0.8	<1.0
1331−234P11	13 31 51.2	−23 25	<0.2	<0.4	1.0	2.2
1331−231P11	13 31 56.4	−23 11	<0.9	<0.5	1.0	2.0
1333−340P11	13 33 01.8	−34 02	0.4	0.7	1.2	1.5
1354−203P11	13 54 33.1	−20 22	<0.6	<0.8	1.5	2.5
1356−188P11	13 56 16.2	−18 48	<0.3	<0.7	1.4	3.6
1402−316P11	14 02 09.7	−31 40	<0.5	<0.3	0.7	<1.5
1404+012P11	14 04 04.9	+01 17	<0.2	<0.5	1.1	<1.2
1423−116P11	14 23 27.8	−11 40	<0.4	<0.4	0.8	1.6
1428−030P11	14 28 51.4	−03 04	<0.2	<0.4	1.0	2.0
1431−326P11	14 31 42.8	−32 37	<0.3	0.4	1.0	<1.2
1444−219P11	14 44 35.4	−21 56	<0.6	<0.4	1.1	1.8
1458−222P11	14 58 56.7	−22 15	<0.3	<0.4	0.8	2.6
1509−211P11	15 09 06.6	−21 07	<0.4	0.7	1.8	2.0
1524+007P11	15 24 04.5	+00 46	<0.2	0.5	1.0	1.5
1548−037P11	15 48 03.4	−03 44	<0.4	0.8	1.3	<1.7
1618+068P11	16 18 30.1	+06 51	<0.3	<0.3	0.7	<1.2
1832−594P11	18 32 32.8	−59 26	0.6	1.5	3.6	5.6
1833−654P11	18 33 21.8	−65 28	0.8	2.5	2.6	<1.7
1840−624P11	18 40 07.9	−62 25	0.4	1.1	2.2	4.4
1844−532P11	18 44 14.7	−53 12	<0.2	<0.4	0.8	<1.7
1919−421P11	19 19 23.9	−42 06	<0.4	<0.5	1.1	<2.1
1955−140P11	19 55 49.9	−14 05	1.0	1.5	1.3	3.1
1958−183P11	19 58 02.7	−18 18	<0.5	<0.7	1.1	1.4
2037−383P11	20 37 58.7	−38 22	<0.4	<0.5	1.5	<2.2

The IRAS fast-moving object search

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The Infrared Astronomical Satellite (IRAS) has provided an all-sky survey at wavelengths between 12 and 100 μm. The satellite also detected fast-moving objects in the Solar System, including new comets and Earth-crossing asteroids, one of which may be an extinct cometary nucleus.

THE first satellite dedicated to astronomical observations at IR wavelengths, the US/Netherlands/UK Infrared Astronomical Satellite (IRAS), was launched on 26 January 1983. The IRAS mission was intended to provide a highly reliable all-sky survey in four wavelength bands centred on 12, 24, 60 and 100 μm (refs 1–4) (These are usually described as bands I, II, III, IV respectively.) The satellite was also able to detect and identify fast-moving objects within the Solar System. In this paper we review the search procedures applied to IRAS data, and the consequent discovery of several new comets and asteroids.

Search strategy

The IRAS survey objectives of high reliability and completeness were achieved by repeated detections in each wavelength band. The detector layout in the focal plane, when combined with overlapping and repeated scans, allowed the positions of sources to be confirmed on time scales of seconds, hours and weeks. This process of repeated confirmation also provides a means of detecting moving objects, as sources which do not repeat at the same celestial coordinates are rejected by the processing software at the ground station. (It should be emphasized that the confirmation process is based on positional agreement, and not flux comparisons between detections; so fixed, but variable, sources are not rejected.)

The sources which were not confirmed during the survey may be divided into three groups.

(1) Seconds confirmation failures. These include objects near to the spacecraft, such as Earth satellites, space debris and material evolved from IRAS; but most seconds confirmation failures are attributable to radiation hits on the detectors.

(2) Hours confirmation failures. These include Solar System objects moving across the sky at more than ~1 arc min h^{-1} (for example, some comets, some main-belt asteroids and most Earth-crossing asteroids).

(3) Weeks confirmation failures. These include slow-moving Solar System objects (such as distant asteroids, comets and planets).

Note that large numbers of detections in band II only, appeared to pass the seconds confirmation test. Examination of the raw data for these detections showed no evidence for the presence of a point source. These apparently spurious point sources should probably be attributed to fluctuations in the diffuse IR background. Chance pairings of these spurious sources were output as possible asteroid candidates. They represented by far the largest group of sources processed by the moving-object software and seriously hindered the search for faint asteroids.

Data from IRAS were transmitted to the Preliminary Analysis Facility (PAF) at the Rutherford Appleton Laboratory in the UK, where examination was confined to seconds and hours confirmation. The raw data were then transmitted to the Science Data Analysis System (SDAS) at the Jet Propulsion Laboratory, California for comprehensive analysis and production of the final data products. Slow-moving Solar System objects which pass both seconds and hours confirmation will not be discovered until analysis is completed at SDAS. Owing to the unavoidable delay in carrying out this analysis, recovery and identification of the many new objects to be found will hardly be possible.

To exploit the potential of IRAS for detecting inner Solar System objects—especially those of low visible, but high IR brightness—appropriate software was developed. An object detected using this software may be described as a 'fast-moving object' (FMO). All sources which passed the seconds, but not the hours, confirmation tests were passed to this FMO detection software.

FMO detection software

The first selection process applied to seconds-confirmed, but hours-failed, sources was based on flux ratios. This was followed by a search for correlations between two comparable detections to provide information on the speed and direction of motion of the source.

The band I/band II and band II/band III flux ratios provide an indication of temperature, and were tested for all multiple-band, non-hours-confirmed detections. To allow for uncertainties in the PAF detector calibrations, these tests were set to pass all sources which gave indicated temperatures between 100 and 400 K. These limits were wide enough to ensure that no true inner Solar System objects were accidentally rejected. Due to source confusion near the galactic plane (which caused many fixed sources to fail hours confirmation), a galactic latitude limit was applied. Objects nearer to the galactic plane than ±5° were rejected.

One consequence of the hours-confirmation strategy (an overlap of half the array on successive orbits) is that a moving object will be observed as two separate non-hours-confirmed detections, if, after one orbit (100 min): (1) it moves more than the position errors due to detector size, and so on, involved in defining 'hours confirmation'; (2) it stays within the telescope field of view.

The minimum motion limit depends on the direction of motion and the number of bands in which the object is detected. In the in-scan direction (approximately along a line of ecliptic longitude), the detector width (for bands I and II) of 0.75 arc min and the satellite scan rate of 3.85 arc min s^{-1} allow motions of ~0.3 arc min per orbit (0.18 arc min h^{-1}) to be recognized. The cross-scan length of the band I and II detectors is 4.5 arc min, but improved resolution is obtained from the offset arrays[4]. Sources detected in more than one wavelength band therefore have more accurately defined positions: thus, smaller motions can be resolved for multiple-band sources. The smallest cross-scan motion which could be recognized was 1 arc min per orbit (0.6 arc min h^{-1}).

The maximum motion which allows the object to remain in the 30-arc min field of view of the telescope depends critically on the component of motion in the cross-scan direction. Sources may have a cross-scan component of motion in the same direction as the scan advance (prograde), of up to 45 arc min per

Table 1 Alerts for which observations were attempted

Candidate	No. of detections	Date	RA	Dec	Magnitude	Observation
180-1	2	25 April	19h 06min	+49°	9	Comet IRAS-Araki-Alcock 1983d
214-1	2	13 May	09 17	−15	17	Comet IRAS 1983f
308-1	3	28 June	01 22	−22	15	Comet IRAS 1983j
315-2	2	2 July	11 03	−32	18	Possible main belt asteroid 1983 NH, lost
333-1	2	11 July	11 57	−49	18	Comet IRAS 1983k
334-1	2	11 July	04 13	−65		No object <18 mag
337-1	2	12 July	01 25	0		No visible objects <18 mag
345-1	2	17 July	01 56	−10		Comet Tempel 2 IR tail
363-1	2	26 July	14 02	−10		Possible Apollo asteroid observed at Palomar—lost
365-1	3	27 July	10 55	−67	17	Comet IRAS 1983o Recovered from
438-1	2	1 September	12 34	−51	17	ephemeris based on IRAS observations
418-1	2	23 August	03 32	+2	16	1983 QF main belt asteroid
460-1	2	12 September	03 54	+2		
424-1	2	25 August	03 28	+31		No object <18 mag
436-1	2	31 August	02 44	−10		1983 QG main belt asteroid
478-1	1	21 September	16 42	−24		Close to new comet Kowal–Vavrova No object seen
516-1	7	11 October	17 30	+59	15.5	1983 TB. Apollo asteroid/inactive comet. Parent body for Geminid meteor stream. Smallest known perihelion distance
559-1	2	1 November	22 10	−58	17	1983 VA. Apollo asteroid
577-1	2	10 November	21 33	−17	15	Comet Hartley–IRAS 1983v

Candidate moving objects were labelled according to the Spacecraft Operating Program (SOP) during which they were detected together with a sequential number within that SOP. Thus IRAS 180-1 was the first moving object alert originating from detections in SOP 180.

orbit under certain circumstances (that is the first detection is on the trailing edge of the array; the second detection is at the leading edge of the array). For the case with retrograde motion the limit is 15 arc min, but it will normally be less.

To minimize chance pairing of sources and to restrict motions to a limit which allowed a reasonable chance of recovery using Schmidt telescopes, the FMO detection software inter-compared all non-hours confirming sources within a search box of specified size. This search box was set throughout the mission to compare all sources within 25 arc min, both in right ascension and declination. Flux comparison tests were applied to pairs of sources separated by one or two orbits which satisfied these motion criteria. To allow for observational errors and possible rotational light-curve variations, flux differences were accepted up to a factor of 6.5 (that is, two magnitudes). All pairs of sources which passed the above tests were output by the software for individual identification. This checking eliminated known asteroids and comets (by comparison with daily ephemerides) and source confusion due to extended sources, such as dark nebulae (by examination of sky survey plates). Single detections of fixed sources which had 'leaked' into the software (for example, those awaiting confirmation on subsequent scans and which had been paired by chance) were also identified at this stage. Candidate moving objects which remained unidentified were communicated, together with an estimated visual magnitude based on the IR fluxes, to cooperating observatories with wide-field cameras for optical confirmation.

The program was first run using IRAS data on 11 February 1983, and, by the end of the month, known main-belt asteroids were being identified. Altogether, 37 alerts were sent to cooperating observatories, of which 17 were not searched for due to unfavourable position, bright moon, or bad weather. The alerts for which observations were attempted are summarized in Table 1. Further details of some strong unobserved or unconfirmed candidates are given in Table 2 for the information of observers who may have exposed plates near to these objects.

IRAS comets

Six comets were discovered by IRAS: the orbital elements derived from IRAS and ground-based measurements are given in Table 3. In addition, five known comets were identified from published ephemerides; data for these are also given in Table 3. The new comets cover a range of inclinations, perihelion distances, and so on, and include both long- and short-period objects. There is a notable tendency for detection to occur at southerly latitudes, but this is more likely to be an effect of the IRAS survey strategy than an indication of the true distribution of cometary orbits. Although it will be necessary to carry out a detailed statistical study to be sure, it is not apparent on an initial examination that comets detected by IRAS have significantly different orbital characteristics from comets detected by observations in the visible.

In terms of individual characteristics, the first comet detected by IRAS (IRAS–Araki–Alcock) was notable because it passed close to the Earth shortly after its discovery, and so was studied in detail both by IRAS and by ground-based observers[5–11]. Dynamically, comets 1983j and 1983v are more interesting. These are short-period comets with inclinations to the ecliptic >45°. Only one of the previously known short-period comets passing perihelion between 1982 and the end of the century (Comet Tuttle) has an inclination of this amount[12]. In fact, Comet 1983v is the only short-period comet with an orbit nearly perpendicular to the ecliptic: it also has the shortest known period for a comet with a retrograde orbit. The question raised by these comets concerns their origin. Assuming that short-period comets are brought about by planetary capture, this can only have occurred when the comets were near the ecliptic plane. For Comet 1983j this is reasonable, because its aphelion distance is very close to the orbit of Saturn.

Comet 1983o was detected by IRAS on 27 July, but was not then recovered by ground-based telescopes. A subsequent IRAS detection on 1 September was recognized, from its IR fluxes, as being a second identification of the same object. An ephemeris based on the two positional measurements was then used to recover the comet from the ground[13]. This seems to be the first occasion on which a comet has been discovered and an ephemeris allowing recovery calculated purely on the basis of satellite observations.

The visual magnitude limit for a comet to be detected by IRAS is estimated to be ≈17. This is based particularly on observations of 1983f and 1983o, which were found to be close to the FMO detection threshold. However, the magnitude limit

Table 2 Strong unobserved or unconfirmed candidates

IRAS candidate	Julian date 244+	RA	Dec	Magnitude	Probable nature
201-1*	5460.11366	08h 40.12min	05° 28.30′	15	Asteroid
	5461.18812	08 40.96	05 26.10		
242-1*	5481.09294	09 46.75	−12 54.90	17	Comet
	5481.52749	09 46.88	−12 54.20		
272-1*	5496.11716	12 03.01	15 00.10	16	Asteroid
	5496.54680	12 03.34	14 55.00		
313-1*	5516.94787	12 21.97	10 48.80	17	Asteroid
	5517.01959	12 22.11	10 47.60		
305-1	5512.79523	13 00.91	15 51.20	17	Asteroid
	5512.86679	13 00.90	15 50.50		
354-1	5537.31041	01 42.46	−10 13.50	18	Apollo asteroid
	5537.38196	01 42.49	−10 12.90		
363-1	5541.93416	14 02.64	−10 22.00	18	Apollo asteroid
	5542.00585	14 02.30	−10 30.70		
429-1	5573.99831	14 12.92	−09 38.00	17	Apollo asteroid
	5574.06992	14 13.20	−09 40.50		
	5574.85718	14 17.48	−10 01.90		
	5574.92879	14 17.76	−10 03.90		
487-1	5603.76624	17 04.34	00 15.40	16	Apollo asteroid
	5603.83779	17 05.42	00 25.60		
	5603.90933	17 06.66	00 35.80		
	5603.98088	17 07.64	00 45.90		
	5603.05242	17 08.80	00 55.90		
576-1	5648.45677	09 29.15	08 12.20	16	Asteroid
	5648.52831	09 29.12	08 11.20		
588-1	5654.46718	10 07.74	07 09.00	17	Apollo asteroid
	5654.53878	10 09.23	07 18.20		

* Discovered during post-mission processing and not communicated to observatories.

Table 3 Orbits for IRAS comet discoveries

Comet	Perihelion time	Perihelion distance (AU)	Eccentricity	Period (yr)	Argument of perihelion (deg)	Longitude of ascending node (deg)	Inclination (deg)	Epoch	Ref.
1983d	21.2529 May 1983	0.991341	0.990115	1004	192.8439	48.4053	73.2495	26 May 1983	Marsden (1983) MPC 8272
1983f	19.0304 January 1983	1.416483	1.0		227.0690	118.9254	152.1948		Marsden (1983) MPC 8052
1983j	23.8021 August 1983	1.696830	0.695573	13.2	356.8916	357.1602	46.1799	14 August 1983	Marsden (1983) MPC 8386
1983k	2.7224 May 1983	2.418226	1.0		265.5959	171.1037	138.8392		Marsden (1983) IAUC 3925
1983o	28.0267 November 1983	2.254646	1.0		333.9918	200.5600	120.7447		Marsden (1983) MPC 8272
1983v	8.5225 January 1984	1.284336	0.836976	22.1	46.9272	0.7738	95.7675		Marsden (1983) MPC 8387
Known comets independently recovered by IRAS									
Pons-Winnecke	7.5035 April 1983	1.253991	0.634714	6.36	172.3194	92.7481	22.3081	15 April 1983	
Tempel-2	1.5355 June 1983	1.381404	0.544893	5.29	190.9220	119.1579	12.4375	25 May 1983	
Tempel-1	9.7951 July 1983	1.491120	0.520897	5.49	179.0424	68.3278	10.5553	4 July 1983	
Kopff	10.3389 August 1983	1.576273	0.544543	6.44	162.8143	120.3074	4.7245	13 August 1983	
Cernis	20.93178 July 1983	3.317031	1.0		186.15198	208.87845	134.69904		Marsden (1983) MPC 8139

Several known comets reaching perihelion in 1983, which may have been detectable by FMO software, were not recovered. These are: Comet Gunn 1969 II; Comet Johnson 1949 II; Comet Taylor 1961 I; Comet Schwassman–Wachmann (1) 1925 II; Comet Bowell–Skiff 1983c*; Comet Shoemaker 1983p; Comet Russell (3) 1983i; Comet Sugano–Saigusa–Fujikawa 1983e. The non-detections are attributable either to motions below the FMO detection threshold, or to the faintness of the object. The exception is Comet 1983e, which was both fast moving and very extended. In this instance, the comet may not have been scanned by IRAS, or have been recognised as a point source.

* Detected as a moving hours-confirmed source at SDAS.

obviously depends on the gas-to-dust ratio, as the IR emission comes from the heated dust. A dusty comet could be detected at somewhat fainter visible magnitudes, whereas a mainly gaseous comet would be visually brighter than the proposed limit. As we have noted, detection of comets is limited not only by brightness, but by motion. Thus Comet 1983t was probably not detected by the FMO software. There has been some confusion on this point as an ambiguous IRAS detection led to a ground-based observation of the comet. However, this was probably a coincidence: not only was the comet of visual magnitude 18 at this time, but its motion (41 arc s h^{-1}) was then below the detection threshold of the FMO software.

There may well be other comets brighter than $M_v = 17$ with motions below 1 arc min h^{-1} in the IRAS database. These were not processed by the FMO software because they passed the hours-confirmation test. It is clearly important to determine whether they will be identifiable during the analysis of slow-moving objects at SDAS. Main-belt asteroids processed by the FMO software exhibited a range of IR flux ratios, due to their range of temperatures and to errors within the IRAS detectors. These were wide enough to encompass all the comet detections except IRAS-Araki-Alcock (whose proximity to Earth resulted in a significantly higher effective temperature). It might therefore appear that moving objects, which are recognized as essentially point sources by IRAS, cannot be separated into comets and asteroids: this has to be done by ground-based observation. In fact, the two classes of objects actually can be distinguished by examination of the unprocessed detector output. As comets are extended sources, the profile of a detection in the in-scan direction is wider than that of a point source. In the cross-scan direction, detectors adjacent to those along the nominal source track may show evidence of a signal, revealing an angular size greater than that of a single detector element. This test was successfully applied to Comet 1983f, which was the faintest of the IRAS comets.

Note that almost all moving, three-band sources detected by the FMO software were identified as known main-belt asteroids. Thus, any such source which cannot be so identified in the IRAS output has a good chance of being a comet. It should be checked for spatial extension by reference to the IRAS unprocessed data.

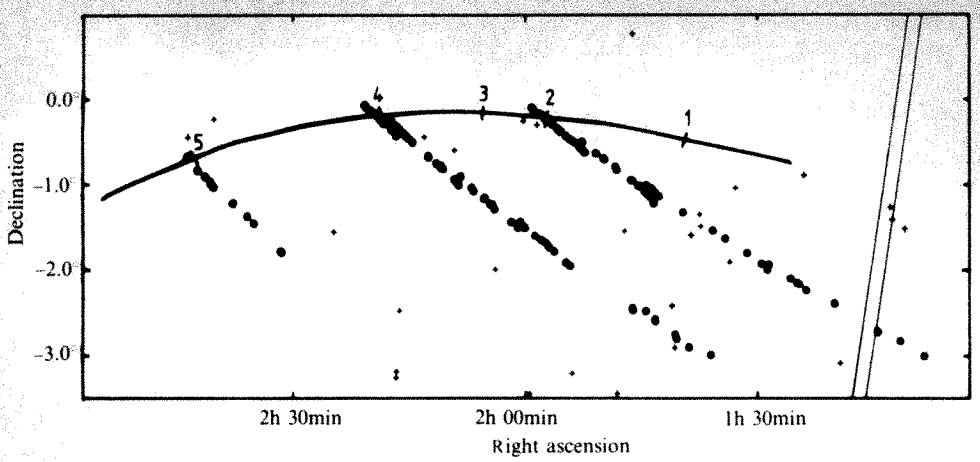


Fig. 1 Positions of non-hours-confirmed sources detected 10–17 July, 21–27 July and 8–9 August. ●, Detections of the tail of Comet Tempel 2; +, other sources. Positions of the comet on (1) 10 July, (2) 17 July, (3) 21 July, (4) 27 July, (5) 9 August, are marked along its track. A single IRAS scan track is also shown at the right-hand side of the diagram.

This should also be done for apparently spurious sources detected during IRAS additional observations: we urge IRAS AO observers to bear this possibility in mind when examining their data.

IR tail of comet Tempel-2

During the period 12–18 July, the FMO software detected about 50 faint, seconds-confirmed sources, having a signal-to-noise ratio of ~5 in the IRAS 25 μm band. These sources seemed to be related to the periodic comet Tempel-2. Figure 1 shows a sample of the detections together with the position of the Tempel-2 nucleus at the times corresponding to their detection. Note that the IRAS scan crosses the tail almost at right angles. With the ~1° day^{-1} progression of the IRAS scan, several days were required to build up a full picture of these features (see Fig. 1). That they represented sections through a continuous tail was verified during the repeat survey scans for weeks confirmation (22–27 July). These showed features in a similar sequence relative to the comet (which had, of course, moved relative to the stars during the intervening period). An examination of the raw data subsequently indicated that the tail was recorded by all band II detectors across the focal plane. Only the most sensitive of these registered a signal-to-noise ratio >5, the PAF processing threshold.

The width of the Tempel-2 tail was found to be ~4 arc min, although its angular length was ~10°. This clearly represents an unusually high aspect ratio. The position of the tail was passed to ground observers, but no optical detection was reported (although a 4 arc min tail was reported by a visual observer[14]).

No similar feature was detected for any of the other comets observed by IRAS. Analysis is under way to determine whether the detection was an effect of optical depth due to the proximity of the Earth to the comet's orbital plane, or whether it was due to an outburst of material in the days preceding the IRAS detection.

Earth-crossing asteroids

It was expected that Earth-approaching asteroids (Apollo asteroids) would dominate the objects discovered by the FMO software. However, as indicated in Table 4, only two IRAS FMO candidates have been optically confirmed as new Apollo objects. In addition, the software independently discovered Apollo asteroid 2201 Oljato, whilst another strong candidate (IRAS 487-1) was not optically recovered.

The small number of detections compared with pre-launch predictions can be attributed to a variety of factors. In particular, the predictions were based on an estimate of the sensitivity in the 12-μm band which proved overoptimistic by approximately a factor of two. In addition, the diffuse 25-μm background, especially near the ecliptic plane, made identification based only on 25-μm detections very uncertain. It should be added that, towards the end of the mission, the scan strategy was changed from coverage of lunes (areas bounded by lines of longitude 60° apart) to scans over the ecliptic poles, resulting in greater sky coverage at high ecliptic latitudes.

Detection of Earth-crossing asteroids also depends critically on their motion. Thus, Apollo asteroid 1620 Geographos was seen only once, and asteroid 1983LC escaped detection totally, because their motions were of the same order as the width of the IRAS scan, but in the opposite direction to that in which the orbital overlap advanced. This allowed them to cross the scanned region between successive IRAS orbits. Conversely, objects whose motions were approximately coincident in direction with the scan advance were detected on several successive orbits, for example 2201 Oljato (six detections), IRAS 487-1 (five detections), 1983TB (seven detections).

As shown in Table 4, the orbital elements of 1983TB are similar to the mean orbital elements of the Geminid meteors[15,16]. The implication is that 1983TB could be the progenitor of the Geminids[17], and may therefore represent a decayed, or inactive, cometary nucleus. No evidence of cometary activity was reported by ground observers, and the source appeared point-like in the IRAS data. The Geminids differ from most meteor showers in the apparently higher mean density of the particles. Hence, we may be seeing here an unusual type of meteor source.

Conclusions

The IRAS mission has provided an all-sky IR survey with a detailed pointing history. The analysis now in progress will allow an estimate to be made of the detectable population of comets

Table 4 Orbital elements of asteroids discovered by IRAS

Asteroid	Absolute magnitude	Epoch	Mean anomaly	Argument of perihelion (deg)	Longitude of ascending node (deg)	Inclination (deg)	Eccentricity	Mean distance (AU)	Ref.
1983 QF	12.5	3 September 83	323.52	277.46	167.34	22.66	0.2164	2.6909	Marsden (1983) MPC* 8207
1983 QG	14.5	27 October 84	98.80	279.55	80.24	14.30	0.3463	2.6430	Marsden (1984) MPC 8467
1983 TB	16.0	27 October 84	290.30	321.69	265.03	22.04	0.8903	1.2715	Marsden (1983) MPC 8394
Geminids	—	—	—	324.8	260.6	23.6	0.896	1.35	I. P. Williams (personal communication)
1983 VA	17.5	2 November 83	349.26	11.74	76.94	16.20	0.6888	2.5821	Marsden (1983) MPC 8395

* Minor planet circulars, Smithsonian Astrophysical Observatory.

and Apollo asteroids. It is, however, possible to draw the following general conclusions from the results already obtained with the FMO programme.

(1) IRAS could detect comets having visual magnitudes <17, provided their motions exceeded ~ 1 arc min h^{-1}.

(2) The positional accuracy of IRAS detections depended on the number of bands in which an object was observed. The accuracy proved adequate for approximate ephemeris calculation, though unsuitable for accurate orbit determination.

(3) There is no evidence for a significant population of undiscovered main belt-asteroids at high ecliptic latitudes, at least amongst those having visual magnitudes brighter than 16 and motions ≥ 1 arc min h^{-1}.

(4) Asteroids and comets are both identified by PAF as point sources. The two types can, however, be distinguished by referring back to the raw data where these are available.

The success of this project would not have been possible without the support and cooperation of the IRAS team at RAL and the help of optical astronomers around the world who reacted promptly to IRAS alerts. We thank especially B. G. Marsden and D. Green of the Central Bureau for Astronomical Telegrams for their invaluable assistance. IRAS is a joint project of US NASA, the Netherlands NIVR and UK SERC. J.K.D. and S.F.G. thank SERC for financial support during this project.

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ARTICLES

Interaction of small and extended defects in nonstoichiometric oxides

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Analyses of the extrinsic and/or intrinsic nature of extended defects in nonstoichiometric titanium and tungsten oxides are made by drawing Burgers' circuits directly onto high-resolution electron micrographs. The essentially interstitial and/or vacancy nature of the small defects responsible for the phases TiO_{2-x} and WO_{3-x}, which exist above the extended defect precipitation temperature, may then be implied. Images suggesting a partial wall of anion vacancy defects have also been obtained.

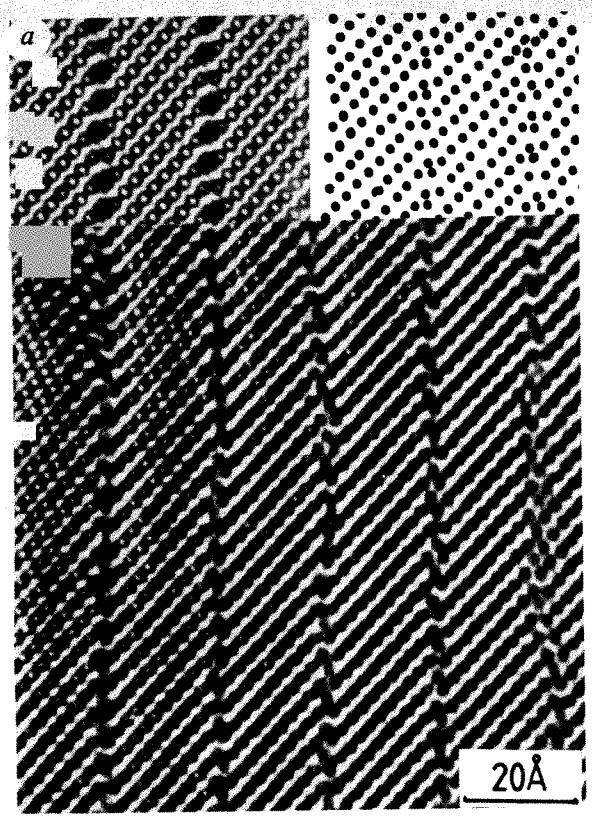
CONSIDERABLE uncertainty and controversy surrounds the nature of the small defects responsible for the nonstoichiometric oxides TiO_{2-x} and WO_{3-x} (refs 1, 2). Thermodynamic studies[3,4] and physical property measurements (such as electrical conductivity[5,6]) are interpreted traditionally in terms of point defect structures; namely, singly- or doubly-ionized oxygen vacancies or cation interstitials. Mechanisms of aggregation or interaction of small defects to precipitate as extended defects have been derived for both oxide systems[7,8], but it has always seemed possible to conceive of alternative or competing mechanisms; one based essentially on charge-compensated oxygen vacancies[9,10], and one based on electrostatically neutral linear cationic interstitial defects[7,8]. We show here that whether crystallographic shear planes (CSP) grow by aggregation of interstitial and/or vacancy defects can be established by determining the extrinsic or intrinsic nature of the partial dislocation bounding the CSP, as revealed by drawing Burgers' circuits directly onto high resolution micrographs. This represents one approach to determining the nature of the small defects.

High resolution electron microscope (HREM) studies of TiO_{2-x} and WO_{3-x} have recently established structural features that were overlooked by earlier lower resolution studies. In the case of slightly-reduced rutile, TiO_{2-x} ($0 \leq x \leq 0.0035$), these include the existence of {100}-platelet defects[11] and the precipitation of pairs of CSP in slowly-cooled samples[12]. In WO_{3-x}, at least three types of extended defects have been observed: CSP[13,14], pentagonal bipyramidal columns (PC)[10,15] and hexagonal tunnels (HTB)[16]. These phenomena were all consistent with new linear interstitial cationic defect models, derived to explain the structure of the small defects in TiO_{2-x} (refs 7, 9).

Experiments

High-resolution images were obtained with the Cambridge University 600-kV HREM[17]. As this microscope has an interpretable resolution limit of better than 2 Å, cation arrangements along CSP can be read directly for appropriately focused images of sufficiently thin crystals[18]. Figures 1*a*, *b* show examples for a [111] specimen of $(Ti, Cr)O_{1.92}$ and a $[100]_{pc}$ specimen of $(W, Nb)O_{2.933}$ respectively. (Further details of specimen preparations and imaging conditions are given in ref 19. Indices without subscripts refer to rutile whereas those subscripted pc refer to pseudocubic axes of WO_{3-x}.) Disorder within the CSP is readily visible in TiO_{2-x} (Fig. 1*a*; see ref. 12 for structural interpretations) and Fig. 1*b* shows evidence for split occupancy of one double-square tunnel (labelled S), corresponding to steps in CSP structure for WO_{3-x} (work in preparation).

Many CSP terminations have been imaged for specimens of $TiO_{1.9966}$ (ref. 19) and $(W, Nb)O_{2.933}$. In each case differences in detail occurred so that it became clear that there was no unique partial dislocation structure. Thus CSP in rutile were often 'decorated' by {100} platelet defects[11,19] (Fig. 2), whereas CSP in tungsten oxides often contained pentagonal columnar



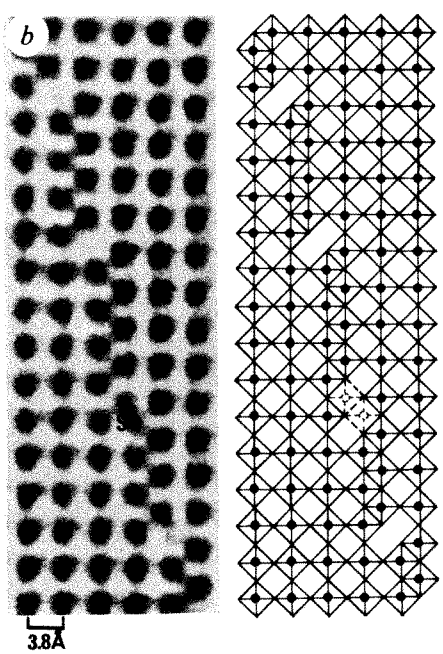


Fig. 1 *a*, 500-kV image of $(Ti, Cr)O_{1.92}$, showing slightly disordered crystallographic shear planes (CSP). Inset shows the structural model for ordered (374) CSP and the computer simulation. Note the sequence of occupied cation sites, which may be read off the image provided the disorder is not excessive. *b*, 500-kV image of $(W, Nb)O_{2.933}$ showing a segment of $(103)_{pc}$ CSP. The inset gives the octahedral representation, showing that tungsten (or Nb) cation positions are located readily by inspection of the image.

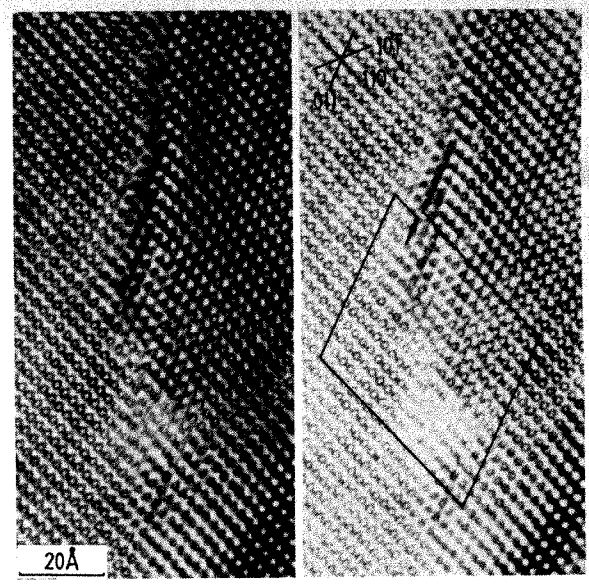


Fig. 2 500-kV image of $TiO_{1.9966}$ (reduced at 1,323 K and cooled slowly over 1 week in a dynamic vacuum of 10^{-6} torr)[11] showing termination of single (132) CSP. The inset shows Burgers' circuit analysis, with closure failure $\sim[0\frac{11}{22}]$, establishing extrinsic nature for this CSP.

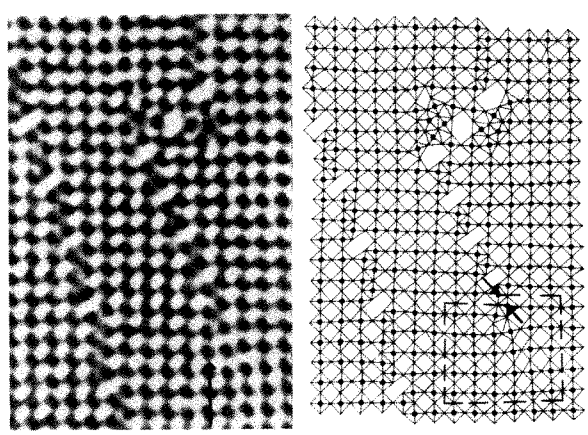


Fig. 3 500-kV image showing termination of $(103)_{pc}$ CSP within WO_3. The location of the terminating half-plane (sight along arrow) establishes the intrinsic nature of this CSP. The inset shows Burgers' circuit analysis, with closure failure $[\frac{11}{22}0]_{pc}$ (see Fig. 2).

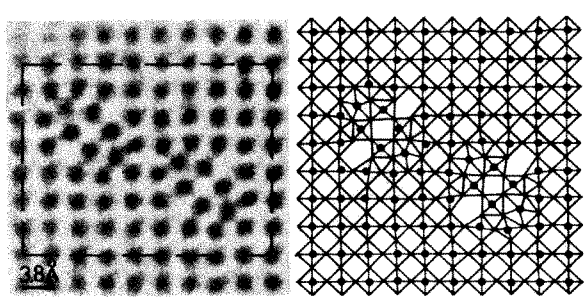


Fig. 4 500-kV image of $(W, Nb)O_{2.933}$ showing two pairs of pentagonal bipyramidal columnar defects; occupied tunnel sites are located by comparison with the structural drawing. Burgers' circuit shows zero lattice displacement.

elements within the termination (Fig. 3). These two examples have been chosen for analysis here because of their relative simplicity; further variations, with structural interpretations, are given in ref. 19.

It was surprising that, for both systems, extended defects occurred which exhibited approximately zero lattice translation, corresponding to pairs of CSP in rutile (see ref. 19) and pairs of occupied pentagonal tunnels for tungsten oxide (Fig. 4).

Burgers' circuit analyses

The geometrical operations for single CSP in TiO_{2-x} and WO_{3-x} may be written $(132)[0\frac{1}{2}\frac{\bar{1}}{2}]$ or $(100)_{pc}[\frac{1}{2}\frac{\bar{1}}{2}0]_{pc}$ for extrinsic, and $(132)[0\frac{\bar{1}}{2}\frac{1}{2}]$ or $(100)_{pc}[\frac{\bar{1}}{2}\frac{1}{2}0]_{pc}$ for intrinsic defects respectively. A convention is adopted that if the CSP has indices (hkl) and displacement $[uvw]$ then the dot product $(hkl)\cdot[uvw]$ is >0 for extrinsic and <0 for intrinsic CSP. In the case of rutile, diffrac-

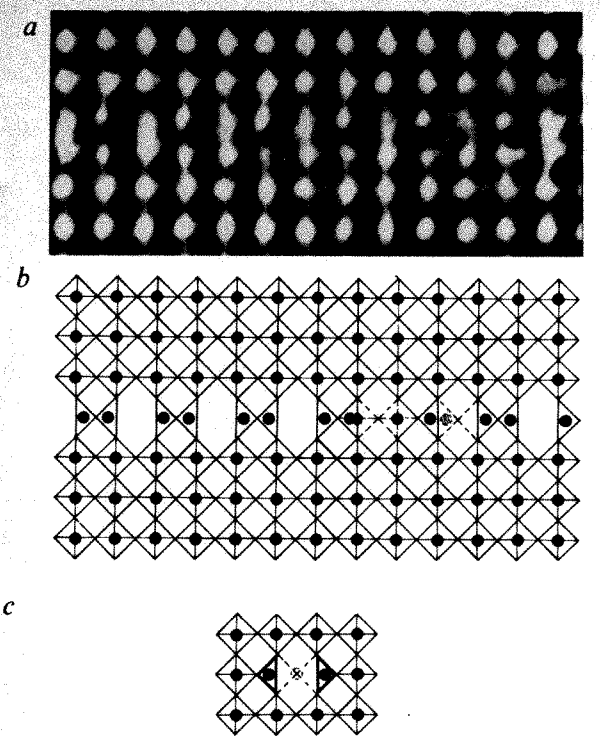


Fig. 5 500-kV image of (W, Nb)$O_{2.933}$ showing novel defect contrast in WO_3 matrix between CSP (*a*), which may be interpreted as a partial wall of oxygen vacancies aligned onto $(100)_{pc}$, premonitory to collapse and formation of a CSP (*b*). Some apparently split-tungsten atom images indicate partial emptying of oxygen sites along projection axis (indicated in *b*). *c*, A structural model for a single oxygen vacancy defect showing relaxation of two adjacent M^{5+} cations into square pyramidal coordination. The relaxation is estimated to be 20% of 3.8 Å (=0.8 Å).

tion contrast experiments gave $[0, \overline{0.45}\cdot 0.45]$, assuming intrinsic CSP[20], and it is the difference between this and the alternative extrinsic vector $[0, 0.55, \overline{0.55}]$ that may be distinguished by direct imaging of the termination. The principles of the Burgers' circuit analysis may be seen by inspection of the drawings given with Figs 2, 3. Thus, starting the Burgers' circuit in a region of perfect crystal, some distance from the defect, and returning to this vicinity, having crossed the single CSP (Figs 2, 3) or circumnavigated the CSP pairs[19] or pentagonal columns (Fig. 4), one finds that a slab of crystal having thickness $(hkl)\cdot[uvw]$ has been added for rutile (Fig. 2) but eliminated for tungsten oxide (Fig. 3) for single CSP, but there is no lattice translation for the defect pairs.

Note that the component of lattice translation parallel to the projection axis is half a lattice vector, for both CSP structures, and is zero for pentagonal columns. Otherwise it would be necessary to image each defect in at least one other projection to measure a third component of the Burgers' vector.

Discussion

It is remarkable that TiO_{2-x} and WO_{3-x} yield opposite characteristics for the terminations of single CSP, implying that the nature of the small defects should be essentially cation interstitial for TiO_{2-x} and essentially oxygen vacancy for WO_{3-x}. For TiO_{2-x} the linear small defect cationic interstitial model[7] also explains the structure and growth of CSP pairs[7,19]. Precipitation of pairs of occupied pentagonal columns in WO_{3-x} is not consistent with oxygen vacancy defects[8], but is explained readily using a linear cationic defect model[21]. Thus the Burgers' circuit shown in Fig. 4 contain two columns having occupancy –O–M–O–M..., in addition to the host WO_3 structure. On the other hand, the presence of single intrinsic CSP in WO_{3-x} implies oxygen vacancy defects.

Strong evidence for the existence of oxygen defects in WO_{3-x} was found inadvertently (Fig. 5*a*). This novel type of defect contrast was located close to the well-known CSP and PC features. Direct image interpretation on the basis of a structural drawing alone, as carried out above (see Figs 3, 4), seems justified although computer simulations will be used to provide further confirmation. Thus the occurrence of double-tunnel contrasts, aligned onto $(100)_{pc}$, with displacements of adjacent black blobs (usually tungsten ion positions), may be interpreted (Fig. 5*b*) as representing a partial wall of oxygen vacancies, accompanied by relaxation of adjacent cations (ideally Nb^{5+} ions) into pairs of adjacent square pyramidal five-coordinated sites. This gives rise to a charge-compensated oxygen vacancy model (Fig. 5*c*). (In practice, at elevated temperatures Nb^{5+} cations may be expected to become detached from the vacancies, being replaced by W^{6+}.) This last observation, together with further results (in preparation), imply that single CSP grow within WO_{3-x} by aggregation of oxygen vacancy defects, either feeding stepwise into existing CSP to be first forming partial walls of oxygen vacancies, premonitory to collapse and production of intrinsic CSP, bounded by a partial dislocation loop (see refs 21, 22).

It would be foolish to claim that in either oxide system, there is a unique CSP, or other extended defect, precipitation mechanism, or that there is only one small defect type in either non-stoichiometric phase. Certainly, in the case of rutile, there appears to be three regimes, where the small defect appears to be predominantly interstitial, predominantly vacancy, or impurity controlled, respectively[5,6,9]. To date HREM analyses have been restricted to specimens cooled from within the interstitial regime. For WO_{3-x} the present observations suggest that oxygen vacancy defects occur, but that interstitial defects may be required for precipitation of PC. It may be that the former occur predominantly on irradiation in the electron beam (see ref. 23) and that specimens cooled slowly across the phase boundary will show evidence for CSP-pair precipitation[8].

Conclusion

The extrinsic or intrinsic nature of extended defects may be determined by drawing Burgers' circuits onto HREM images. Such analyses may also yield, by implication, the nature of small defects existing within nonstoichiometric phases just before precipitation. It seems unlikely that unique and reproducible CSP partial dislocation structures occur in either TiO_{2-x} or WO_{3-x}, the structure of individual defects being dependent on the relative concentrations of different small defect types existing locally at the time of precipitation. Further attempts to obtain specimens and images from different regions spanning the non-stoichiometric phases are planned, but this will require even greater control of specimen preparation conditions than used so far, especially for WO_{3-x} where the phase limits are poorly defined[4].

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Somatic recombination in a murine T-cell receptor gene

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A putative T-cell receptor gene was isolated from the DNA of the helper hybridoma, 2B4. Analysis of the sequence components of this rearranged gene indicates the presence of separate variable (V), *diversity* (D) *and joining* (J) *region elements analogous to those of heavy-chain immunoglobulins. These findings further support the belief that the products of this locus are involved in antigen recognition by T cells. There is no evidence of somatic mutation between the putative germ-line and the expressed variable-region gene.*

T LYMPHOCYTES have a variety of important roles in the immune system of higher organisms, including the direct killing of virally infected cells (cytotoxic T cells) and either amplification (helper T cells) or inhibition (suppressor T cells) of the response to antigen. In order for T cells to perform these functions, they require a receptor for antigen which allows them to distinguish 'self' from 'non-self'. While the T-cell receptor appears similar to that of B cells (antibody) in that it is clonally distributed and has a wide range of specificities, it is functionally distinct in that it can only recognize antigen in association with a product of the major histocompatibility complex (MHC): the *H-2* region in the mouse and the *HLA* region in man. Particular subsets of T cells use different MHC molecules; cytotoxic T cells require class I molecules (H-2K, D, L in the mouse) and helper T cells require class II molecules (H-2A and E in the mouse). Any structural model of the T-cell receptors(s) must explain how these types of T cell can recognize antigen only in conjunction with self-MHC, yet respond strongly to MHC determinants from other individuals (alloreactivity) without previous exposure to these antigens, and how the ability to 'learn' self-MHC occurs in the thymus, also independent of antigen. There are two major schools of thought which attempt to explain these phenomena: the 'one receptor' hypotheses[1] hold that T cells recognize a complex of antigen plus MHC ('altered self') while the 'two receptor' models[2] propose separate receptors for antigen and MHC ('dual recognition'). Recent evidence from cloned T-cell lines and hybridomas strongly favours the view that the receptor(s) cannot recognize antigen or MHC independently[3-5], although there are contradictory data from other sources[6,7].

Recently, Hedrick *et al.*[8] isolated a cDNA clone (TM86) which is T cell-specific, localized on membrane-bound polysomes and systematically rearranged in a variety of T-cell lines and hybridomas. Sequence analysis of this and related cDNA clones (86T1, 86T3 and 86T5) indicates significant similarity to immunoglobulin variable and constant regions and an independently assorting 16-amino acid element homologous to both heavy- and light-chain J regions[9]. Antisera raised against synthetic peptide fragments of this molecule significantly inhibit the *in vitro* response of certain T-helper (T_H) hybridomas (J. Rothbard *et al.*, in preparation). Thus, it seems probable that these cDNAs encode one chain of the murine T-cell antigen receptor. Other workers[10] recently isolated a cDNA clone from a human T-cell lymphoma line which appears to encode a closely related molecule. To investigate the structure of the genes encoding these molecules and the rearrangements that occur at the genomic level during T-cell differentiation, we examined the pigeon cytochrome *c*-specific, H-2E-restricted T_H hybridoma, 2B4 (ref. 11). A monoclonal antibody specific to this particular line has been isolated and shown to precipitate a 43,000 (43K)-molecular weight disulphide-linked heterodimer[11] characteristic of the murine receptor for antigen[12,13].

Sequence of a 2B4 cDNA clone

A library of cDNA clones from 2B4 mRNA was constructed as described elsewhere[14] in the vector λgt-10 (ref. 15). Phage were screened with 86T5, a cDNA clone largely containing constant region sequences of the putative receptor molecule[9]. Approximately 0.02% of the clones were positive, of which two distinct types were evident: one of these derives from the fusion partner BW5147 and will be described elsewhere—the other type appears to be specific to the 2B4 hybridoma and the structure and sequence of a representative clone, p2B4.71, is shown in Fig. 1. The amino acid numbering system is that of the mature protein, assuming a signal peptide cleavage 19 amino acids from the initiation condon[9] (by analogy to immunoglobulin). Unlike the previously described murine cDNAs[9], p2B4.71 possesses a complete 3′ end with a stop codon after amino acid 287 and a 3′-untranslated region of 174 nucleotides, ending with a poly(A) addition signal 17 nucleotides 5′ to the poly(A) tail. The 5′ end is not complete as shown, but we were subsequently able to deduce the remaining variable region–leader polypeptide sequence from the genomic clones (see below).

The amino-terminal portion of the constant region (amino acids 115–241) has only one nucleotide change (silent) at amino acid 201. However, after position 241, the nucleotide sequences diverge significantly (15%) (Fig. 1). Interestingly, the amino acid sequence is relatively unchanged (only four amino acid changes result from 22 nucleotide changes). Although these differences could have been due to divergence between strains of mice in a single gene (86T1 is from BALB/c and p2B4.71 is from B10.A), our recent data indicate that the constant regions of these cDNAs are derived from two different genes and that the regions of similarity and divergence are encoded in separate exons[42].

Also interesting is the fact that the same *J* region used in TM86 (ref. 9) appears again in the 2B4.71 sequence but joined to a different variable region, further confirming the ability of these elements to assort independently of the variable regions. TM86 derives from a T_H hybridoma, 3.3T (ref. 4), which responds to a cytochrome *c* fragment different from that of 2B4, but using the same MHC backgrounds (B10.A (2R) for 3.3T and B10.A for 2B4). While the 5′ regions do not appear to be similar, it may be significant that they share the same *J* region. In contrast to the TM86 fragment, the p2B4.71 variable region shows significant similarity to that of 86T1, which was obtained randomly from a thymocyte library[9]. Homologous residues are indicated by asterisks in Fig. 1. In all, 31/90 (34%) residues in the portion of variable region shown are identical; most of these are located in the same regions that are relatively invariant (framework residues) in both heavy- and light-chain immunoglobulins[16]. Although it will be necessary to sequence many more T-cell variable regions to be certain, this comparison indicates that the structure of the receptor binding site may be very similar to

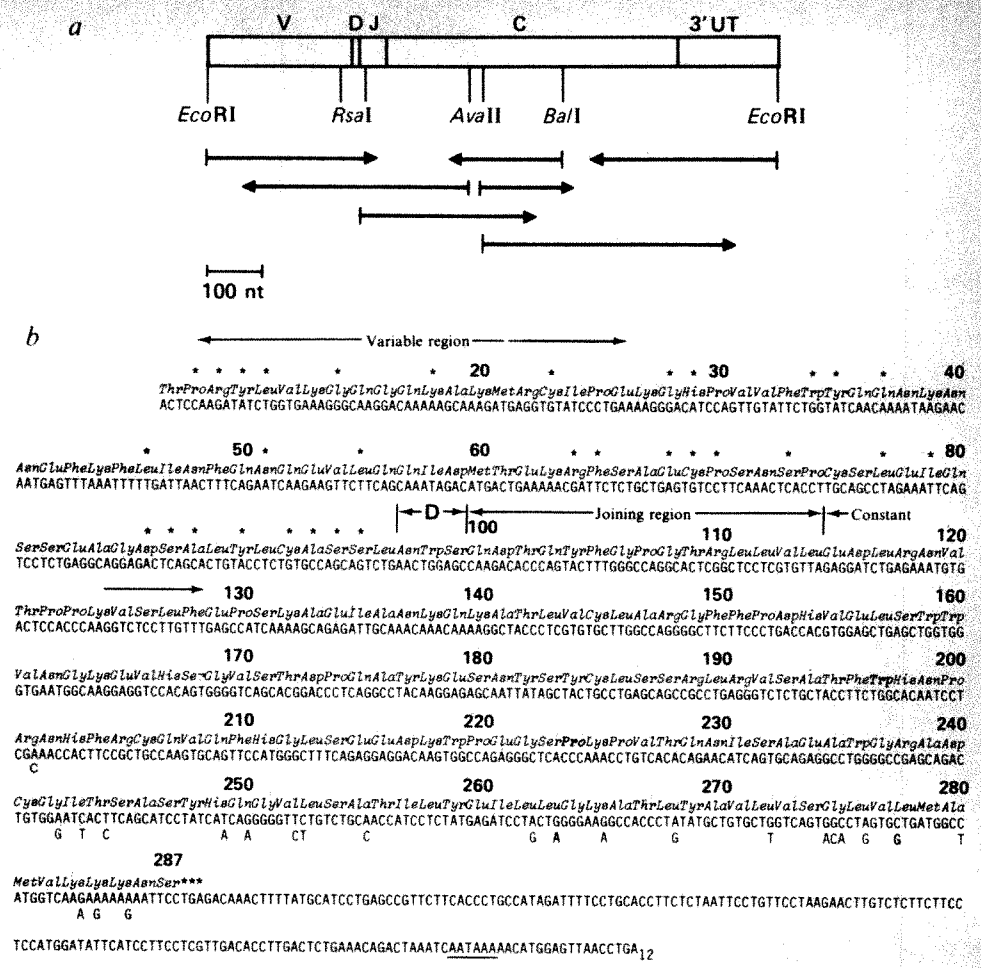


Fig. 1 Sequence of 2B4.71.A cDNA library of ~200,000 recombinants was constructed as described[14] using poly(A)$^+$ cytoplasmic RNA from the T-helper hybridoma, 2B4. The library was screened by standard procedures and positive clones subcloned into pUC9 (ref. 18) for further characterization. One such clone, p2B4.71, was sequenced using the procedures of Maxam and Gilbert[19]. The sequencing strategy is shown in *a*. The sequence starts after the *Eco*RI linker site. Residues marked by asterisks are those homologous to the variable region of 86T1. Nucleotides beneath the constant-region sequence represent differences compared with the constant-region sequence of 86T1.

that of immunoglobulins with respect to framework and complementarity determining residues.

Corresponding genomic clones

To characterize the gene which gave rise to the p2B4.71 cDNA clone, as well as to reconstruct the recombinational events which must have occurred in its formation, we prepared λ genomic libraries[17] of both 2B4 tumour DNA and B10.A liver DNA. The 2B4 library was screened with constant-region probes and the liver libraries were screened with separate variable- and constant-region probes. Figure 2 shows the restriction maps of the resulting clones. The B.6 clone contains an unrearranged (presumably germ-line) variable region from the B10.A genomic library. Three overlapping clones (B.21, B.22 and B.3) containing constant-region genes were isolated from the liver DNA library. Southern blotting analysis showed that all the constant-region genes in the mouse genome are contained within this 25-kilobase (kb) region. Further analysis showed that these consist of two constant-region genes, each with a distinct *J*-region cluster[42]. Two sets of genomic clones were isolated from the 2B4 hybridoma library. One set represents the result of an apparent rearrangement and will be described elsewhere. The other set, represented by 2B4.1 and 2B4.9, encode all of the gene segments of p2B4.71. As with the case of immunoglobulins, the rearranged genes appear to result from the recombination of a variable region and its 5′ flanking sequences with a *J*–*C* locus and its 3′ flanking sequences, as indicated by the shading in Fig. 2. The homologies indicated were determined by heteroduplex analysis (data not shown), restriction mapping and sequence analysis.

Variable region formation

In order to analyse the assembly of the variable-region exon, we subcloned the relevant restriction fragments from λ clones 2B4.9, B.21, B.22 and B.6 into the plasmid vector pUC9 (ref. 18) and sequenced them by the procedure of Maxam and Gilbert[19]. Figure 3*a*, *b* indicates the sequencing strategy for the rearranged variable-region fragment and the corresponding liver segments. The strategy used to sequence the *J* region is shown in Fig. 4 as part of the larger region (see below). The sequences themselves are shown in Fig. 3*c*, together with the predicted amino acid residues. The indicated leader polypeptide exon is deduced from the very close similarity that the 2B4 sequence

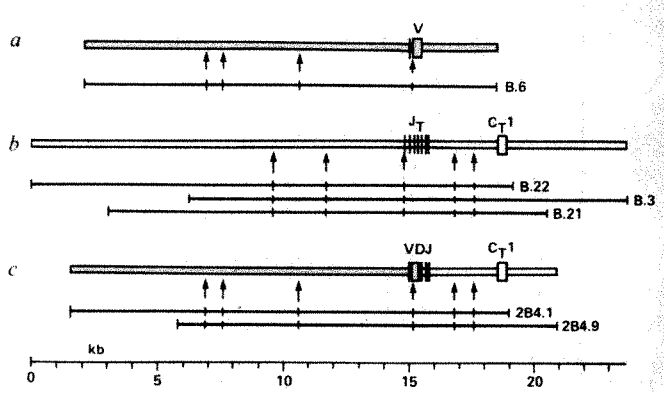


Fig. 2 Genomic clones from liver and hybridoma libraries. Shading indicates similarities between clones as determined by restriction mapping and heteroduplex analysis. ↑, *Eco*RI sites. Assignment of variable (*V*) and joining (*J*) elements is based on the sequence analysis and that of the first constant-region exon[42]. All clones are shown 5′ to 3′ with respect to the coding regions. B10.A liver and 2B4 T-helper hybridoma DNAs were partially digested with *Mbo*I, size-selected on sucrose gradients and ligated to *Bam*HI-digested arms of the λ vector J1 (a derivative of L47.1) (ref. 39) and packaged *in vitro* as described elsewhere[17]. The T_H hybridoma library was screened with a predominantly constant-region probe (86T5)[9]. The liver DNA libraries were screened with both constant-(86T5) and variable-region probes from p2B4.71.

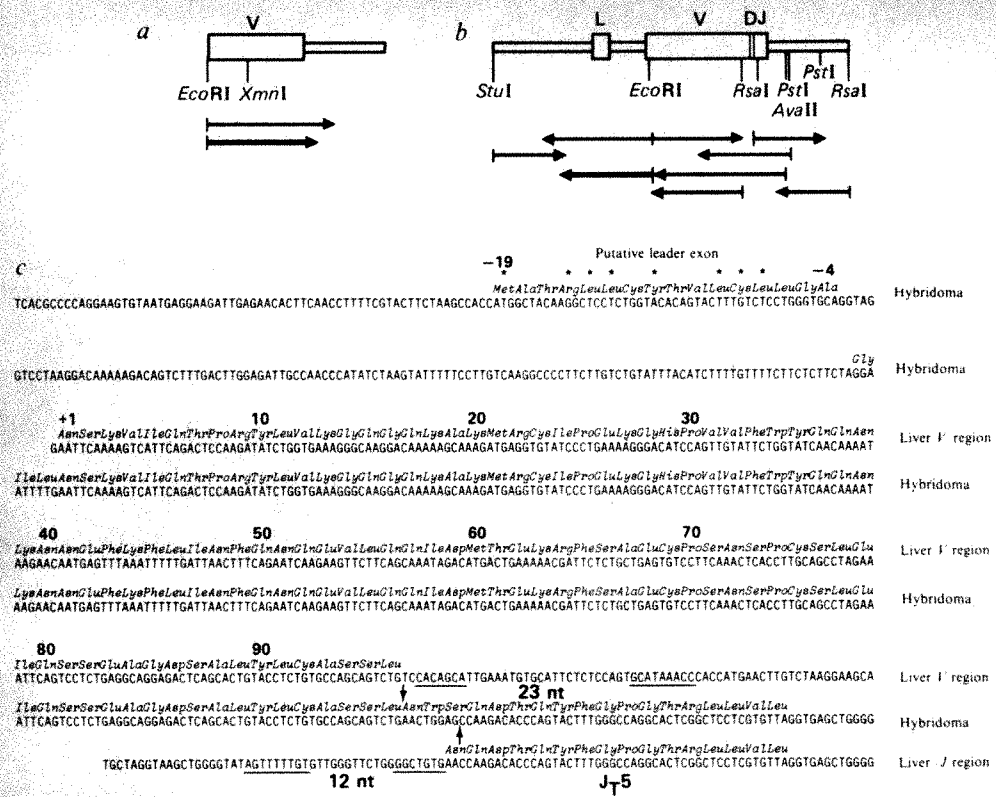


Fig. 3 Assembly of the 2B4 variable region. The sequencing strategy for the liver (*a*) and 2B4 (*b*) derived variable-region fragments. The *J*-region sequence is taken from the data presented in Fig. 4. Thin lines indicate 5′-end labelling with polynucleotide kinase and thick lines indicate 3′-end labelling with the large (Klenow) fragment of DNA polymerase I[19]. The sequences are shown in *c*, with the apparent leader exon (residues homologous to the 86T1 leader are marked with asterisks) and the variable, diversity and joining region components as indicated. Putative signal sequences for DNA rearrangement, separated by 23 and 12 nucleotides (nt) respectively, are underlined. Arrows indicate the apparent points of rearrangement.

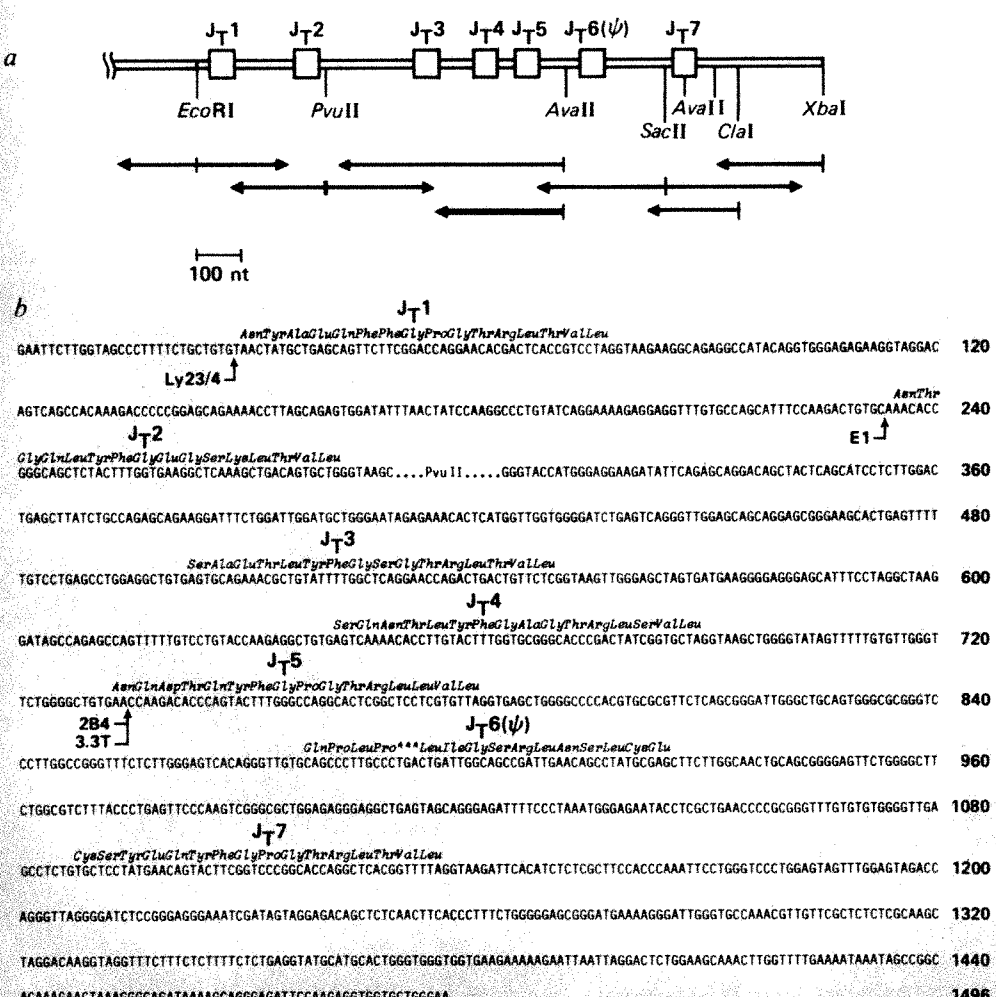


Fig. 4 *J*-region cluster. The fragment indicated from the genomic clone B.22 was sequenced by the strategy shown in *a*. Thin lines indicate 5′-end labelling and thick lines represent 3′-end labelling. Apparent J_T elements were identified based on amino acid and flanking sequence homologies to expressed J_T regions[9]. The stop codon in the J_T6 pseudogene is indicated by ***. The apparent points of rearrangement of p2B4.71(2B4) and of TM86[9] (3.3T) as well as those of cDNA clones derived from E1 (ref. 40) and Ly 23/4 (ref. 41 and P. Patten *et al.*, unpublished results) are indicated by arrows.

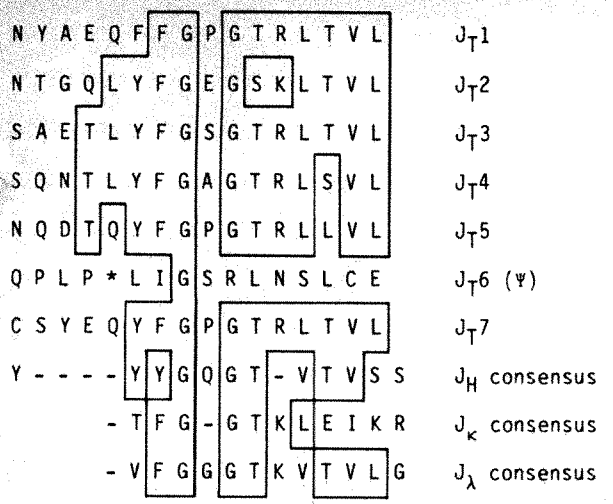


Fig. 5 Amino acid homologies between the seven *J*-region elements. Comparison of the coding sequence homologies between the *J* regions and the consensus sequences from the three different types of immunoglobulin[16].

as to that of 86T1 (ref. 9) (8/15) residues at identical positions). This assignment is probably correct because of the virtual identity that these presumptive intron–exon boundaries have with immunoglobulins[20].

Figure 3*c* also shows that an 8-nucleotide sequence at the 3′ portion of the rearranged variable region is not derived from either of the two liver DNA sequences. This sequence is at a position similar to the *D* or diversity region in immunoglobulin heavy chains and codes for a similar number of residues[21–25]. Although we cannot exclude the possibility that this extra sequence is not encoded in the germ line[26], it does bear an intriguing resemblance to that of an immunoglobulin *D* element, Q52 (CAACTGGAC)[22]. As recent data show that this gene is not located on the same chromosome as the mouse heavy-chain locus[43], this homology might derive from some functional constraints on the *D* region common to both the T-cell receptor and immunoglobulins. The existence of a family of such elements for the T-cell molecule could greatly increase the number of possible variable regions by at least three mechanisms known from the example of immunoglobulin genes: combinatorial association of a third genetic element[21], variability in the junction points at the DNA level[21,23], and the possibility of reading in different frames[27].

It is important to compare the unrearranged *V* region with the corresponding 294 nucleotides of the hybridoma gene. Within this region there is complete homology between the hybridoma and liver-derived gene sequences. This indicates that no somatic mutation has occurred between the putative germline and the active gene, and while this does not rule out a role for such mutation in other contexts, it does make some of the more extreme models of receptor adaptation to self-MHC and antigen less likely[28].

Also significant is the fact that by combining the complete, processed *V(DJ)* region sequence shown in Fig. 3*c* with the complete constant region of Fig. 1*b*, we can deduce a molecular weight of 32.1K. This is in very close agreement with the value of 31K obtained in tunicamycin studies of the unglycosylated murine receptor peptides[29].

Directly 3′ to the *V* region of the germ-line DNA in Fig. 3*c* is the sequence CACAGCA-[23 nucleotides]-GCATAAACC which is similar to the putative signal sequences for gene segment fusion 3′ to the immunoglobulin *V* regions[30,31]. In immunoglobulins, the sequence is typically CACAGTG-[11 or 22 nucleotides]-ACAAAAACC 3′ to the *V*[22,27]; the inverse of the putative *J*-region signal sequences (see below). Although the analysis of one *V* region is insufficient to establish exact sequence requirements, it seems significant that the last two nucleotides of the putative *V*-region heptamer (CA) and the first nucleotide of the nonamer (G) have never appeared in those positions of the relevant immunoglobulin sequences. These similarities and differences compared with immunoglobulin flanking sequences are discussed more extensively with the *J*-region data below.

J-region cluster

Sequence analysis of the liver DNA-derived clones in the region immediately surrounding the point of rearrangement (Fig. 1) indicates the presence of a cluster of seven *J* regions. Three of these *J* regions (J_T1, J_T2 and J_T5) have been found expressed in different cDNA clones (see Fig. 4 legend), and as many as six may be functional. J_T6 is clearly not functional because of the in-frame stop codon. Figure 4 shows the sequencing strategy of the 1.8-kb fragment containing the *J* regions. The J_T cluster begins ~3.6 kb 5′ to the first constant-region domain[42]. The J_T elements are very closely spaced at 40–220 nucleotides apart, whereas immunoglobulin *J* regions are typically 300–500 nucleotides apart[22,30,31]. The *J* region used by p2B4.71 and TM86 (ref. 9) is the fifth in the cluster (J_T5) and, as indicated in Fig. 4, joining occurs at the same position for each. Of particular interest are the putative signal sequences for gene segment fusion 5′ to each *J* region. In immunoglobulins, these sequences, spaced either 10–12 or 21–23 nucleotides apart, are highly conserved between all functional *J* regions in a variety of species[21,22,30,31]. The sequence is typically GGTTTTTGT-[11 or 22 nucleotides]-CACTGTG 5′ to *J* or *D* elements and the inverse: CACAGTG-[11 or 22 nucleotides]-ACAAAAACC 3′ to *V*. The sequence 5′ to three of the *J* regions in Fig. 4 is AGTTTTTGT-[12 nucleotides]-GGCTGTG. The other sequences diverge slightly from this consensus. Although these sequences are very similar to those of the immunoglobulin genes in all cases, the heptamer is not palindromic and the sequence 'GG' has not been found in any of the many immunoglobulin sequences in this position in either mouse, human or rat[16]. The region immediately 3′ to the apparent functional *J* regions also contains conserved sequences, as indicated in Fig. 4, but these may have more to do with RNA splicing than recombination. We conclude that the gene segments examined here have sequences which are analogous to those used by immunoglobulins for variable-region assembly but that they differ slightly in the conserved sequence,

9	(10–14)	7	J_T coding regions	
ATTCTTGGT	AGCCCTTTTC	TGCTGTG	TAACTATGCTGAGCAGTTCTTCGGACCAGGAACACGACTCACCGTCCTAG GTAAGA	J_T1
GAGGTTTGT	GCCAGC(AT)TTCCAA	GACTGTG	CAAACACCGGGCAGCTCTACTTTGGTGAAGGCTCAAAGCTGACAGTGCTGG GTAAGC	J_T2
AGTTTTTGT	CCTGAGCCTGGA	GGCTGTG	AGTGCAGAAACGCTGTATTTTGGCTCAGGAACCAGACTGACTGTTCTCG GTAAGT	J_T3
AGTTTTTGT	CCTGTACCAAGA	GGCTGTG	AGTCAAAACACCTTGTACTTTGGTGCGGGCACCCGACTATCGGTGCTAG GTAAGC	J_T4
AGTTTTTGT	GTTGGGTTCTGG	GGCTGTG	AACCAAGACACCCAGTACTTTGGGCCAGGCACTCGGCTCCTCGTGTTAG GTGAGC	J_T5
CGGGTTTCT	CTTGGGTCACAG	GGTTGTG	CAGCCCTTGCCCTGACTGATTGGCAGCCGATTGAACAGCCTATGCGAGC TTCTTG	J_T6(Ψ)
CGGGTTTGT	GTGTGGGGTTGA	GCCTCTG	TGCTCCTATGAACAGTACTTCGGTCCCGGCACCAGGCTCACGGTTTTAG GTAAGA	J_T7

Fig. 6 Flanking sequence homologies among *J* regions both 5′ and 3′ (see text); homologies are underlined.

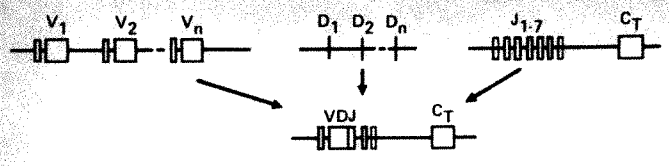


Fig. 7 Model of 2B4 T-cell receptor gene rearrangement. Variable (*V*), diversity (*D*) and joining (*J*) region elements all appear to contribute to the formation of the 2B4 gene examined here.

perhaps to allow independent regulation of rearrangement in T and B cells. Another possibility is that there is no functional difference between the flanking sequences of the T-cell *J* regions and those of immunoglobulins; this would account for the non-functional *D–J* joining found in some T-cell clones[25] and would suggest that the usual B cell/T cell specificity of rearrangement is dependent on other signals. No immunoglobulin light-chain rearrangement has been observed in T cells and we have not seen any rearrangement of the T-cell locus discussed here in three different B-cell lymphoma lines, nor in a macrophage line.

Figure 5 illustrates the homology between *J* regions of the cluster described here in the potential coding residues. As with *J* regions in any specific immunoglobulin family, all except the pseudo-*J* are highly homologous. Note also that the previously identified (from cDNA clones) 86T1/86T5 *J* region[9] is not within this cluster, nor is another *J* region found in a cDNA clones from a concanavalin A-stimulated spleen cell library (unpublished results). These and other J_T regions appear to reside in a second cluster 5′ to the one examined here[42].

Implications for antigen recognition

The T-cell gene studied here bears a remarkable resemblance to those of immunoglobulins in amino acid sequence, patterns of rearrangement and the sizes and positions of the different gene segments. Even the flanking sequences relevant to rearrangement differ only slightly. These similarities must reflect the common origin of these genes in evolution as well as the strength of the selective pressures involved. Also interesting is the complexity of the rearrangements (see Fig. 7); the formation of the variable-region exon requires as many gene segments (*V*, *D* and *J*) as the most complex of the immunoglobulins. This is in contrast to the expectation that the T-cell receptor should represent a simpler type of gene (perhaps like light-chain immunoglobulins) because cell-mediated immunity may have arisen earlier in evolution than humoral (antibody-mediated) immunity. Instead, it seems that the T-cell gene is at least as complex as the most elaborate of the immunoglobulin genes and that T-cell recognition of antigen and MHC may involve a range of specificities equivalent to that of immunoglobulins. It is also interesting to note that the '11-22' rule[21] which in B-cell heavy chains may only permit *V* and *J* segments to join to *D* (and not to each other) does not exclude direct *V–J* joining in the example seen here. If such direct joining is possible, it would provide additional flexibility in the formation of the variable region of this molecule.

The similarities to immunoglobulins noted above seem to place considerable constraints on the structure of the second chain of the receptor heterodimer. The intertwining of the heavy- and light-chain polypeptides with each other in immunoglobulins as defined by X-ray crystallography[32,33] makes it unlikely that one chain could be so similar to immunoglobulin and the other chain very different. This idea is reinforced by the peptide mapping data indicating variable and constant portions of both chains[34–36]. Therefore, one would expect the second chain to be very similar to the one described here and that the combining site formed must resemble that of immunoglobulin. Indeed, even the possible hypervariable and framework residues seen in the comparison with 86T1 and p2B4.71 (Fig. 1) fall into almost the same positions as those of immunoglobulin. This favours the one-receptor model of antigen–MHC recognition, but introduces the unexpected paradox of how to account for all the additional requirements of T-cell recognition and thymic learning in a molecule that is as yet indistinguishable from immunoglobulins. One possibility is that a low-affinity interaction with self-MHC occurs in the thymus which eliminates T cells which either cannot bind to self-MHC at all, or bind too tightly. Evidence has been obtained for the existence of highly self-MHC-reactive cells being present in, but never leaving the thymus (B. Benaceraf, personal communication). Perhaps only those cells with a low affinity for self-MHC can emerge from the thymus and are available to recognize antigen–MHC complexes, which they presumably do at some higher affinity. Alternatively, the receptor molecule could be largely used in binding antigen while the MHC interaction depends more on accessory molecules such as L3T4 in helper T cells[37] and Lyt 2,3 in cytotoxic T cells[38]. The availability of cloned probes and detailed structural data should considerably aid experiments designed to test this and other models.

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Kinked DNA in crystalline complex with *Eco*RI endonuclease

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The 3 Å electron density map of a co-crystalline recognition complex between Eco*RI endonuclease and the oligonucleotide TCGCGAATTCGCG reveals that a tight, complementary interface between the enzyme and the major groove of the DNA is the major determinant of sequence specificity. The DNA contains a torsional kink and other departures from the B conformation which unwind the DNA and thereby widen the major groove in the recognition site.*

THE interactions between proteins and specific DNA sequences are key regulatory events in the control of many biological processes. A full understanding of the recognition mechanisms which determine these interactions requires detailed knowledge of the structure of representative DNA–protein interfaces. This knowledge may also reveal general recognition rules which could govern a broad range of DNA–protein interactions. Recently, the crystal structures of several proteins which recognize specific sites on DNA have been solved[1–5]. In the absence of DNA–protein co-crystals of high quality, these protein structures alone have provided the basis for extensive model building and speculation concerning the appearance of the recognition complexes[1–8]. However, these studies are limited by the necessary assumption that significant conformational changes do not occur in either the protein or the DNA. Similarly, crystallographic studies on B-DNA have revealed detailed information on the structure of DNA in the absence of protein[9–14]. We report here our preliminary observations of the first sequence-specific DNA–protein complex to be visualized by X-ray diffraction methods: *Eco*RI endonuclease complexed with the synthetic oligonucleotide TCGCGAATTCGCG (*Eco*RI site underlined).

Solution of the structure

*Eco*RI endonuclease and TCGCGAATTCGCG co-crystallize in space group P321 with one 32,000-molecular weight enzyme subunit and one strand of DNA in the asymmetric unit, as reported previously[15]. The crystals were grown in the absence of Mg^{2+}, which is required for cleavage of the DNA. Data were collected by oscillation film photography as described previously[15] on the native crystal and on platinum and mercury derivatives. The platinum derivative was obtained by soaking crystals in 10 mM *cis*-$(NH_3)_2PtCl_2$, dissolved in 40 mM bis-tris-propane (BTP), *p*H 7.0, plus a mixture of 11% w/v polyethylene glycol (PEG) 6000 and 5% w/v PEG 400 for 7 days at 25 °C. The Hg derivative was similarly obtained by soaking crystals in 5 mM $Hg(SCH_2CH_2NH_3^+)_2$, dissolved in 40 mM BTP, *p*H 7.0, plus 16% w/v PEG 6000 for 3.5 days at 25 °C. The heavy atom locations were independently determined by difference Patterson methods for both derivatives. (Even though the platinum compound is a chemotherapeutic agent which appears to act at the DNA level, the location of the major and minor sites are found on the surface of the protein, well away from the DNA.) The Cullis *R* factors for the Pt and Hg derivatives to 3.5 Å are 49.2% and 52.2%, respectively.

A multiple isomorphous replacement (MIR) electron density map was calculated from these two derivatives to 5 Å resolution. In addition, Wang's iterative single isomorphous replacement (ISIR) method (B.-C.W., in preparation) was independently applied to the Pt and Hg data. This procedure identifies protein and solvent regions in a SIR electron density map and uses this information to resolve the SIR phase ambiguity. The general features, such as the solvent regions and the molecular outline, were similar in all three electron density maps; however, the MIR and Hg-ISIR maps contained significant amounts of noise, while the Pt-ISIR map was very clear. We suspect that the noise is associated with non-isomorphism in the Hg derivative (even the Pt derivative was not strictly isomorphic beyond 3.5 Å). We therefore used the ISIR method to resolve the phase ambiguity in the 3.5 Å Pt SIR phases. The ISIR method was also used to extend the phase information in successive stages to 3.2 and 3.0 Å resolution. The mean figures of merit for the 3.5 and 3.0 Å phases are 0.78 and 0.79, respectively. Electron density maps calculated with the 3.5 and 3.0 Å Pt-ISIR phases were very similar apart from the expected greater detail in the higher resolution map. Both maps showed clearly recognizable features, including the α-helix seen in Fig. 1. The enantiomorph was chosen so that α-helices were right handed.

The DNA was easily located in the 3.0 Å map and coordinates were obtained for the centroids of all 12 phosphate moieties, the 13 sugars and 12 of the bases. These coordinates enabled us to calculate the general DNA conformational parameters via the helix determination procedures developed in conjunction with the ApU and GpC structures[16] as well as to construct Figs 2 and 3.

Structural features

Inspection of the Pt-ISIR electron density map revealed several striking features which were clear before a complete tracing of the polypeptide chain and fitting of molecular models. These broad features are discussed below.

The DNA–*Eco*RI endonuclease complex is a symmetrical globular structure ~50 Å across (Fig. 2). The molecular boun-

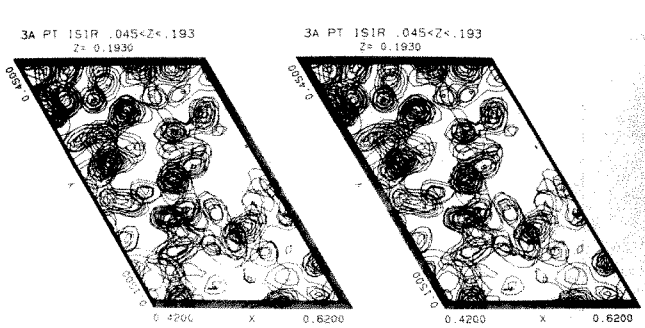


Fig. 1 A representative α-helix. A stereo pair showing a portion of the 3.0 Å ISIR electron density map illustrating the general quality of the map. An α-helix runs vertically up the page (this is not the α-helix discussed in the text which interacts with the DNA).

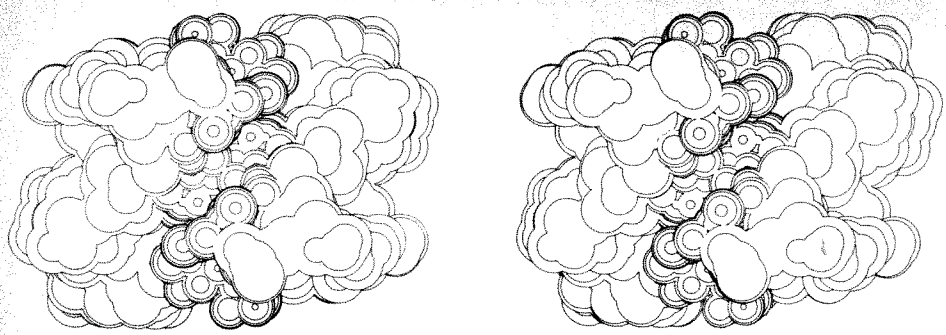


Fig. 2 The DNA–*Eco*RI endonuclease complex. This schematic representation of the surface of the DNA–protein complex was constructed by superimposing spheres on the electron density map so that their surfaces followed the molecular boundary. The DNA is shaded more darkly than the protein. The complex is viewed from the solvent region, looking down the crystallographic 2-fold symmetry axis. The DNA is clearly embedded in the protein and the active sites for recognition and hydrolysis are underneath the DNA.

dary clearly encloses a well defined complex consisting of two enzyme subunits and one double helix. The two protein subunits and the two strands of DNA are related by a common crystallographic axis of 2-fold symmetry, as expected from the contents of the asymmetric unit[15].

The packing of these complexes within the crystalline lattice is typical of that generally seen in macromolecular crystals, that is, a limited number of fortuitous interactions between symmetry-related complexes. One of these is a DNA–DNA interaction in which symmetry-related double helices are packed end to end, forming a continuous rod of DNA parallel to the *c*-axis. This is one of the common modes of packing found in crystals containing only DNA and probably contributes to the relatively strong diffraction pattern of this particular crystalline form of *Eco*RI endonuclease. The unpaired thymine moieties at each end of a double helix appear to be in contact with thymine residues from symmetry-related double helices.

The DNA conformation departs from the classical B-motif (Fig. 3). The 'distortions' are not uniformly distributed over the entire DNA molecule; rather they are concentrated in three abrupt transitions termed kinks. These kinks create four blocks of three base pairs each:

TCGC-GAA-TTC-GCG
GCG-CTT-AAG-CGCT

where the kinks are indicated by dashes. These features arise from systematic shifts in the coordinates of all the phosphate, sugar and base moieties in each intervening 3-base pair (bp) segment, that is, they represent local adjustments to net displacements of entire blocks of double helical DNA. These kinks are conceptually related to the model structures originally predicted by Crick and Klug[17], although they differ significantly in detail. Additional kinked models were proposed by Sobell *et al.*[18,19] and by Zhurkin *et al.*[20].

We suggest a flexible nomenclature for the DNA conformation because subsequent DNA–protein structures will probably reveal additional kinks and other novel DNA features. We refer to the overall DNA conformation as 'neo RI-DNA'. The specific context of neo relates to a DNA conformation which is stabilized by the binding of a recognition protein.

The left-most block will be referred to as the terminal or CGC block and the next block as the central or GAA block. The two blocks on the right (TTC and GCG) are identical to the respective left blocks because of the 2-fold symmetry axis.

We designate the transition between the symmetry-related central blocks (GAA–TTC) as the neo-1 kink and that between the terminal and central blocks (CGC–GAA) as the neo-2 kink. Neo-2 kinks appear twice within the structure (CGC–GAA and TTC–GCG) and both are identical by virtue of the crystallographic axis of 2-fold symmetry.

When the helical symmetry within each block was determined by analysing the geometrical relationships of the centroids of the phosphate and internal deoxyribose moieties according to the procedure of Rosenberg *et al.*[16], B-type parameters were found for the central block: 10.3 residues per turn with a helical displacement of 3.2 Å. The values for the terminal block appear more like those usually associated with A-DNA: 11.3 residues per turn and 2.7 Å per residue. The location of the helix axes and the apparent tilt of the bases with respect to these axes are also suggestive of B- and A-DNA in the two respective blocks.

We emphasize that we are not claiming that the blocks between kinks are identical to either 'classical' A- or B-DNA. Indeed, these designations must be considered tentative until our analysis has reached the point that sugar pucker and other detailed conformational parameters can be determined unambiguously. We also suspect that relatively smaller structural departures from exact helical symmetry will be noted within the blocks once the structure is carefully refined.

Comparison with other DNA structures

The DNA in our co-crystal is basically the dodecamer studied extensively by Dickerson and colleagues[9–14,21] with the addition of a 5'-thymidylate residue. The kinks described here differ significantly from the features reported by Dickerson *et al.*, suggesting that the neo RI-DNA structure is not stable in the absence of the enzyme. This does not exclude the possibility that the neo RI conformation exists transiently due to thermal fluctuations nor does it exclude the possibility that these transient states are kinetic intermediates of complex formation.

Dickerson's dodecamer structure also possesses A-like features in the region we have described as the CGC block[10]. This led Dickerson and Drew to suggest that *Eco*RI sites are B-like within the canonical hexanucleotide flanked by A-like regions[10]. Our structure appears to confirm this suggestion and to indicate that this trend is amplified in the protein–DNA complex.

The 10.3 bp per turn helical repeat of the central blocks is within the values of 10.0 to 10.6 bp per turn reported for DNA in solution[22,23], bound on crystalline surfaces[24,25] and in nucleosomes[26,27].

Nature of the kinks

The neo-1 kink, at the centre of Fig. 2, enlarges the grooves, thereby facilitating access by the protein to the bases. This kink is a prominent feature of Fig. 3*b*, where the backbone can be seen to 'jog' upward in the centre of the figure. The displacement increases the distance between those segments of DNA backbone which straddle the kink by ~4 Å. This is achieved by a systematic unwinding of the entire DNA molecule by ~25°, that is, the neo-1 kink is primarily a torsional dislocation of the double helix.

The neo-1 kink introduces a small bend in the DNA such that the helices of the symmetry-related central blocks are inclined at an angle of ~12° to each other. (This value is preliminary and it is anticipated that model fitting and refinement will result in adjustments to its value.) The bend is towards the minor groove, as suggested by Crick and Klug[17].

The neo-2 kink is different in several respects from neo-1. The neo-1 possesses 2-fold symmetry while the neo-2 kink is asymmetrical. The neo-2 kink does not involve a dramatic unwinding of the DNA; however, it introduces a larger bend of ~23° between the helix axes. This bend includes rolling motion towards the minor groove. The neo-2 kink may also represent an A–B junction.

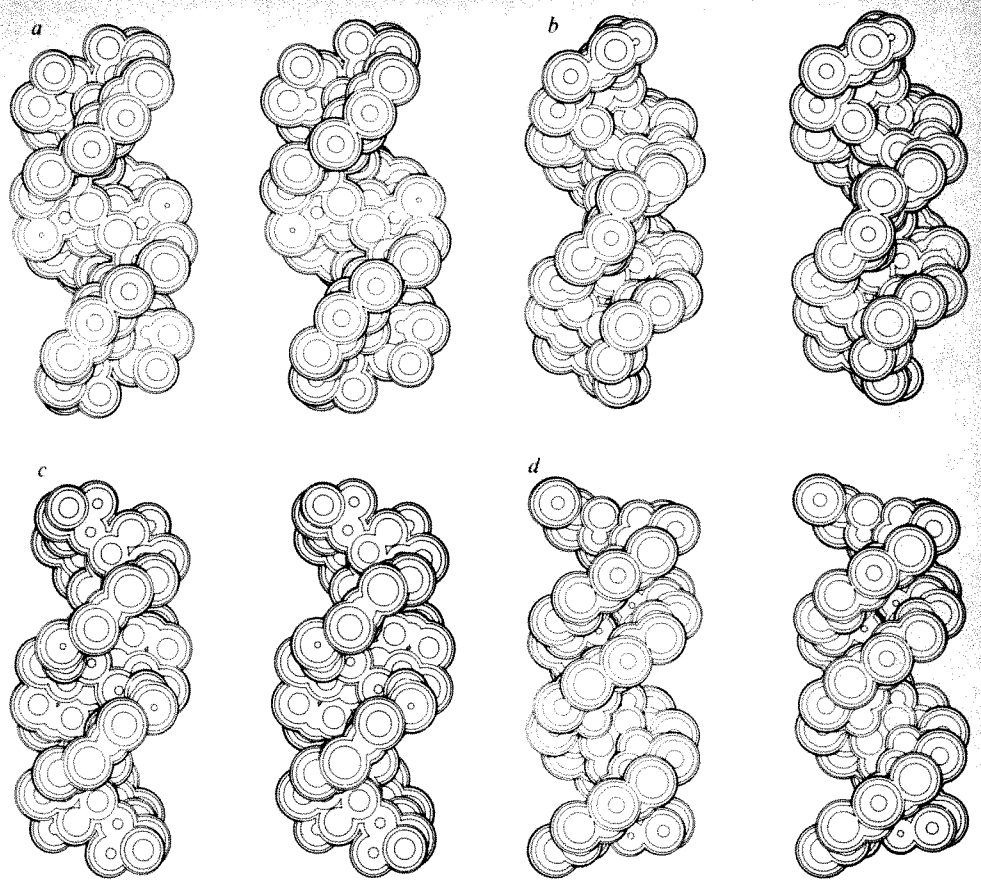


Fig. 3 The neo RI-DNA conformation. *a*, A stereo representation of the portion of the electron density corresponding to the DNA produced by placing spheres at the approximate positions of the phosphate, deoxyribose and base moieties (radii of 4, 3.5 and 3.5 Å, respectively). The view is from within the protein, towards the major groove where the sequence-specific recognition contacts are concentrated. The two parallel segments of DNA backbone running across the front of the figure are buried in clefts in the enzyme and include the phosphodiester linkages which would be cleaved in the presence of Mg^{2+}. *b*, The DNA rotated by 90° about the average helix axis. The neo-1 kink, which is between the two central blocks, can be seen in the centre. Note also the expansion of the major groove (which opens towards the left in the upper portion of the figure). *c*, The DNA rotated by 180° from that shown in *a*, that is, viewed from the solvent region as in Fig. 2. Kinks between the terminal blocks of 3 bp and the middle blocks can be noted in the backbone segments closest to the viewer. They are of the type described as neo-2 in the text. *d*, A similar representation of the B-DNA model which Arnott and co-workers[50] derived from fibre diffraction data.

Recent crystallographic observations of DNA structural variability *in vitro* have led to suggestions that DNA *in vivo* is structurally heterogeneous, and that structural variations may be associated with sequence-specific DNA–protein recognition[10,28]. The structural results include major departures from B-DNA, such as Z-DNA[28–31] as well as relatively smaller local variations within the B-motif[10,14,32,33]. The association between protein recognition and altered DNA conformation has been enhanced by the observation that DNA sequences which have the potential to form Z-DNA can be found within certain eukaryotic regulatory elements[34,35].

The kinks described here represent additional experimentally observed elements of DNA structural heterogeneity which are clearly associated with the binding of a recognition protein. The neo-1 kink is an essential element of the recognition event itself because the symmetry-paired α-helices (see below) cannot fit into the major groove unless that groove has been expanded via the neo-1 kink. This does not exclude an additional involvement of the kinks, especially the neo-2 kink, in the strand scission event also. This raises the expectation that DNA–protein recognition in general will turn out to be associated with specific deviations from the average B-double helix. The neo-1 and neo-2 kinks are probably general structural features that will be found in other DNA recognition systems. However, these generalizations cannot yet be established since this is the first X-ray structure of DNA in intimate association with a sequence-specific recognition protein.

DNA–protein interaction

The major groove of the recognition hexanucleotide (GAATTC) appears to be filled with protein, forming a large, complementary interface. All the base–amino acid interactions appear to be located in the major groove of the DNA, while the edges of the bases which are exposed in the minor groove are open to the solvent. Thus, the primary determinant of the recognition specificity is the direct interaction of complementary surfaces in the major groove. However, additional features of this structure raise the possibility of a second, indirect mechanism whereby sequences flanking the canonical hexanucleotide modulate the binding (see below).

A major component of the DNA–protein interaction is an α-helix which makes an angle of ~60° with the average DNA helix axis. There are two such α-helical structures, side by side, related by the crystallographic 2-fold symmetry axis. The polypeptide chain turns sharply at the end of each α-helix, leaving the amino acid residues at the end of the helix, in the bend and those in the adjacent stretch of chain in close proximity to the DNA.

Two symmetry-related pairs of amino acid side chains appear to be in contact with symmetry-related pairs of sequential adenine residues. These amino acid side chains are located in the α-helix-bend motifs described above, although each of the two physically adjacent side chains originates from a different protein subunit.

Flanking the recognition interface are two symmetry-related DNA backbone segments, those nearest the viewer in Fig. 3*a*, which are deeply buried in symmetrical clefts in the enzyme. These clefts extend from the bottom of the wide semi-cylindrical depression which contains the DNA, giving the protein a 'threaded' appearance. The buried backbone segments include the scissile phosphodiester bonds.

While the two 'arms' of protein enveloping the DNA from the upper left and lower right of Fig. 2 appear to originate in the underlying subunits, they might alternatively originate from symmetry-related molecules which are nearby due to crystal packing. We favour the interpretation that they are part of the underlying subunits because the electron density maps show extensive continuity with the underlying electron density and very limited continuity with the nearby, symmetry-related electron density. Furthermore, we have a provisional tracing of the polypeptide chain in this region in which each arm is a loop of protein which emerges from the underlying region. However, we emphasize that we cannot entirely exclude the alternative until we have an unambiguous tracing of the entire polypeptide chain.

If these arms do extend from the underlying protein, this would necessitate a conformational change in the enzyme during the complete catalytic cycle because the DNA could not get past the arms and enter the active site unless something moves. Such a conformational change has been implied by some of our biochemical results (ref. 36 and L.J.-J. *et al.*, unpublished). The conformational change could arise from two possibilities which are not mutually exclusive: mobility of the arms themselves and quaternary changes in which the two subunits in the dimer move with respect to each other.

Comparisons with inferred contact points

The DNA contact points described above show both similarities and differences when compared with the contacts inferred by Lu *et al.*[37] from the results of alkylation interference and protection experiments. They suggested contacts in the major groove (at the recognition guanine N^7 position) as well as phosphate contacts in good general agreement with our structure. Lu *et al.* also made two observations regarding the N^3 position of the central adenine, which is in the minor groove. This site was protected from dimethylsulphate by endonuclease, and prior methylation at this point blocked subsequent binding of the enzyme. They suggested that *Eco*RI endonuclease is in physical contact with the central adenine at N^3 (however, they also indicated that other interpretations were possible). We do not see any interaction between the protein and DNA at that position. This methylation site is in the region of the DNA most affected by the neo-1 kink and base–base steric effects could be responsible for these methylation results. Lu *et al.* also observed that while the N^3 of the adjacent adenine was protected by the endonuclease, prior methylation at this second point did not block subsequent binding of the enzyme. The factors responsible for this result are unknown, although we do not see any minor groove DNA–protein interactions there either. We therefore conclude that while alkylation protection and interference data can be used to infer a good low resolution indication of the location of the DNA–protein contacts, they cannot be reliably used to infer the precise points of DNA–protein interaction.

Another approach used to identify contacts on the DNA is based on the hydrogen bonding degeneracy rules of Seeman *et al.*[38–40]. In *Eco*RI endonuclease such degeneracy can be induced by conditions of elevated *p*H (8–9.5), Mn^{2+}, low ionic strength and by the addition of organic compounds such as glycerol or ethylene glycol[41–45]. This has been called the *Eco*RI* reaction[41] and the non-canonical sites so recognized are referred to as *Eco*RI* sites. We showed that hierarchies of base preferences could be discerned by qualitative estimations of the rate of cleavage at *Eco*RI* sites in plasmid DNA molecules. The observed hierarchies are uniquely predicted by a recognition model which assumes two major groove hydrogen bonds per base pair in standard conditions[40]. One or more of these are randomly replaced by a water–DNA hydrogen bond in *Eco*RI* conditions. The position of any *Eco*RI* sequence within the appropriate hierarchy is primarily determined by the number of remaining protein–DNA hydrogen bonds[40]. Woodbury *et al.*[39] reached similar conclusions from a somewhat different perspective. The major groove recognition points inferred from these analyses are in excellent agreement with our X-ray structure. Thus, degeneracy analysis appears to be a promising method for the study of DNA–protein interactions.

Flanking sequence effect

Thomas and Davis[46] established that DNA sequences flanking the recognition site influence the hydrolysis rate by approximately an order of magnitude. These results have been confirmed and extended[47–49]. Two hypotheses (which are not mutually exclusive) can be advanced to explain these data: (1) Contacts occur between the enzyme and DNA bases outside the canonical hexanucleotide; (2) the conformational free energy of the neo-2 kink and/or of the terminal block depends on the flanking base sequence.

We have seen no significant interactions between *Eco*RI endonuclease and bases outside the canonical hexanucleotide. However, we must again emphasize that this cannot be stated unequivocally until all relevant side chains have been located and their positions refined. We do see substantial interactions between the protein and DNA backbone segments which extend beyond the canonical hexanucleotide. These observations are consistent with the latter hypothesis.

This raises the possibility that *Eco*RI endonuclease interacts with DNA in two sequence-dependent ways: (1) A direct specificity interaction with the central hexanucleotide which creates the precise requirement for the *Eco*RI sequence, GAATTC; (2) an indirect interaction with sequence 'selectivity' which subtly modulates the binding energy as a function of base sequence within the regions immediately flanking the *Eco*RI site. Sequence specificity is mediated by physical contact, such as hydrogen bonds, between the protein and DNA bases. Sequence selectivity may be indirectly mediated by sequence-dependent DNA conformational forces.

Conclusions

The following observations include those which we feel are most significant: (1) The direct sequence-specific DNA–protein interactions involve the major groove of the DNA in a tight, complementary interface with *Eco*RI endonuclease; (2) minor groove contacts do not appear to be significant determinants of sequence specificity in this case; (3) the DNA conformation in the recognition complex is a departure from the B-motif; (4) the torsional neo-1 kink is required for the protein to fit into the major groove of the DNA; (5) kinks are now experimentally observed elements of DNA structural heterogeneity which may have a general role in DNA–protein recognition.

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LETTERS TO NATURE

Reverse stellar evolution, quasars and low-mass X-ray binaries

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Recently, Mathews has proposed[1] a mechanism for the mass supply to a black hole in the centre of a quasar (see also ref. 2). According to this proposal the X rays from the central source penetrate the photosphere surrounding low-mass stars. The absorbed energy is distributed over the stellar interior by convection, and the star expands rapidly. Its outer layers are then stripped by the radiation field of the central source. There are two major uncertainties in this model of reversed evolution; the depth to which the X rays impinging on a low-mass star can penetrate before they are absorbed and the inward transport of the energy flux. The crucial hypothesis of Mathews is that the X rays are absorbed within the convection zone. We now test this hypothesis by considering the effects of X-ray absorption in the secondaries of low-mass X-ray binaries. We conclude that reverse evolution does not occur.

In low-mass X-ray binaries a late dwarf transfers matter by Roche-lobe overflow to a neutron star through an accretion disk[3]. As generally no X-ray eclipses in these systems are observed, it has been concluded that the secondaries are shielded from the X rays of the neutron star by the accretion disk[4]. Optical studies have shown that this shielding is not complete. Orbital variations in the optical, due to X-ray heating of the surface of the secondary facing the neutron star, have been detected in Sco X-1, 4U2129+47 and 4U/MXB1735−44 (ref. 5). In Sco X-1 and 4U1735−44 part of these variations may be due to heating of a bulge in the accretion disk[5]. The recently discovered sharp X-ray eclipses in MXB1659−29 (ref. 6) show that the secondary in this system intercepts X rays from the neutron star. Optical pulses have been detected in 4U1626–67 at a sidelobe frequency to that of the X-ray pulses and shown to arise from X-ray reprocessing on the secondary. The strength of the optical pulses shows that a large fraction of the surface of the secondary facing the neutron star is exposed to the X rays[7]. The importance of these observations for the present discussion is that they show that the secondaries in low-mass X-ray binaries are exposed to X-ray irradiation, and that reverse evolution—if viable—should occur in them. In particular, we note that a main sequence companion star to 4U1626–67 would be $\sim 0.08\ M_\odot$ (ref. 7), and therefore precisely the kind of red dwarf considered by Mathews[1].

We will compare the time scale of the evolution of an X-ray binary undergoing reverse stellar evolution with the Kelvin–Helmholtz time scale of the mass-losing secondary

$$\tau_{KH} = \frac{GM^2}{RL} \simeq 3\times 10^7\ \mathrm{yr}\left(\frac{M_\odot}{M}\right)^2 \qquad (1)$$

Here M and R are the mass and radius of the secondary, respectively. To obtain the last equality we have used the mass–radius and mass–luminosity relations for the main sequence $R \propto M$ and $L \propto M^3$. For fully convective low-mass stars the Kelvin–Helmholtz time scale is of the order of 10^9 yr.

Let us suppose that the X-ray energy flux were transported inwards to heat the interior directly. The star would then adjust its structure on a time scale given by

$$\tau_X = \tau_{KH}\frac{L}{L_a} \qquad (2)$$

where $L_a (\gg L)$ is the absorbed luminosity. A single star in the X-radiation field of a quasar would expand on the time scale given by equation (2), which is much shorter than the Kelvin–Helmholtz time scale. In a low-mass binary the expansion would lead to mass-transfer on the expansion time scale τ_X.

To estimate the relevant time scales we have to determine the amount of absorbed X-ray energy, which in the binary can be written

$$L_a = f\frac{\pi R^2}{4\pi a^2}L_X \simeq \frac{f}{4}\left(\frac{R}{a}\right)^2\frac{L_x}{L_{Edd}}1.8\times 10^{38}\ \mathrm{erg\ s^{-1}} \qquad (3)$$

Here a is the semi-major axis of the binary orbit, L_X is the X-ray luminosity and L_{Edd} is the Eddington luminosity of a $1.4\text{-}M_\odot$ neutron star, equal to $1.8\times 10^{38}\ \mathrm{erg\ s^{-1}}$. f is the product of a correction factor to the rough estimate $(R/a)^2/4$ of the spatial angle subtended by the secondary as seen from the neutron star, the fraction of this spatial angle that is not shielded by the accretion disk, and the fraction of the X rays impinging on the surface that penetrate into the stellar interior. Assuming with Mathews that approximately half of the X-ray flux penetrates deeply, we find typical values for f betweeen a fifth and a tenth. (R/a) is a slowly varying function of the secondary mass, well approximated by[8]

$$\frac{R}{a} \simeq 0.46\left(\frac{M}{M+M_{ns}}\right)^{1/3} \qquad (4)$$

Combining equations (1)–(4) we get

$$\tau_X \simeq \frac{1.3\times 10^4}{f}\ \mathrm{yr}\ \frac{M}{M_\odot}\left(\frac{M+M_{ns}}{M}\right)^{2/3}\frac{L_{Edd}}{L_X} \qquad (5)$$

If reverse evolution could operate, the following would happen in a low-mass X-ray binary. Once the mass transfer starts, the X rays will penetrate into the secondary and cause it to expand. The expansion will increase the mass transfer, and hence cause a higher X-ray luminosity. Thus the mass transfer and the X-ray luminosity grow exponentially, until the Eddington luminosity is reached, or until the mass flow prevents the radiation produced by the accretion process reaching the convection zone of the secondary. After this the evolution proceeds linearly on the time scale given by equation (5), that is shorter than $\sim 10^5$ yr. The initial exponential growth of the mass transfer rate occurs in the time $\tau_x h/R$ needed by the secondary to expand an atmospheric pressure scale-height h outside its Roche lobe. This is within $\sim 10^4$ yr, even for low-luminosity X-ray binaries ($L_x \simeq 10^{36}\ \mathrm{erg\ s^{-1}}$).

The consequences for our Galaxy are then that most low-mass X-ray binaries should be at the Eddington limit, and presumably many of them would show strong absorption effects in the X rays. This is not supported by the observations. Only if the shielding of the secondary by the accretion disk and the scattering of the hard X rays reduces f below $\sim 10^{-4}$ could reverse evolution be compatible with the observations.

In addition, if low-mass binaries were undergoing reverse evolution their average life time would be of the order of 10^5 yr

only. The impossibility of such short life times is particularly clear in globular clusters. The number of bright low-mass X-ray binaries observed in each of the densest globular clusters is of order unity[9]. For an average life time of τ yr this means that a system has to be formed in a typical dense cluster every $1/\tau$ yr. The formation rate[10] as calculated from the number of close encounters between compact remnants and main sequence stars in the cluster is in good agreement with an average life time of the low-mass X-ray binary of $\sim 10^9$ yr, but not with a life time of 10^5 yr.

We note that a secondary in a low-mass X-ray binary receives an X-ray flux $\geqslant 10^4$ times that of a low-mass star in the X-ray radiation field of a quasar. The X-ray flux received by the latter is more comparable with that received by a secondary in a cataclysmic variable. There is no evidence for reverse evolution in cataclysmic variables.

We have offered empirical evidence that reverse evolution does not occur. But we must not overlook the severe theoretical problems with this idea. An intense flux of incident radiation would tend to quench convection in the outer layers; even if free energy were somehow available to maintain the star in a well-mixed (constant entropy) state, thermodynamical arguments would still restrict the efficiency of inward energy transport against the temperature gradient.

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The nature of low-frequency cutoffs in the radio emission spectra of pulsars

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Low-frequency cutoffs were first observed in the radiation spectra of several pulsars about a decade ago[1]. The detailed study of the low-frequency ($\omega \leqslant 100$ MHz) radio emission spectra of more than 50 pulsars[2–7] has shown that practically all pulsars observed have low-frequency cutoffs in their spectra. In most pulsars the spectral cutoffs are an inherent feature of pulsar emission and are obviously associated with the emission mechanism[8]. The most favoured hypothesis at present is that the radio emission of pulsars is a curvature radiation of charged plasma bunches that move, with a Lorentz factor of $\Gamma \cong 10^3$, practically along curved magnetic field lines[9,10]. The kinetic energy of plasma bunches is comparable with the brightness temperature of radio emission measured in energy units. Hence, the reabsorption of the curvature radiation by the radiating plasma bunches themselves may be essential for the formation of the radio emission spectra of pulsars. Here we show that this radiation reabsorption may be responsible for low-frequency cutoffs in the pulsar radio emission spectra.

The curvature radiation of a system of charged ultrarelativistic particles ejected from the polar cap of a pulsar with a dipole magnetic field has been discussed in detail elsewhere[11], taking into account the emission reabsorption by radiating particles. The spectral density of the curvature radiation flux $F(\vartheta_K, \omega)$ was derived for the direction forming an angle ϑ_K with the magnetic axis at a frequency ω. Following ref. 11 we consider the case of a monoenergetic distribution of ejected particles and the power dependence of their density at the pulsar surface on the distance d to the pulsar's magnetic axis:

$$f(\Gamma, \vartheta_0) = K_0 \delta(\Gamma - \Gamma_0)\left(\frac{\lambda}{\vartheta_0}\right)^{\beta} \Theta[\vartheta_0 - \lambda]\Theta[\hat{\vartheta}_0 - \vartheta_0]$$

where $f(\Gamma, \vartheta_0)$ is the distribution function of ejected particles, K_0 and β are constants, $\vartheta_0 = \arcsin(d/R)$ is the angular coordinate of points on the pulsar surface, measured from its magnetic axis, $\Theta[x]$ is the step function equal to unity for $x \geqslant 0$ and to zero for $x < 0$, and $\hat{\vartheta}_0 \simeq (\Omega R/c)^{1/2}$ is the angular radius of the polar cap of the pulsar, separating the open magnetic field lines, along which particles are ejected ($\vartheta_0 < \hat{\vartheta}_0$), from the closed lines, along which no plasma ejection occurs ($\vartheta_0 > \hat{\vartheta}_0$); $\lambda \simeq \hat{\vartheta}_0^2$; Ω is the angular rotation velocity of the pulsar and R is its radius.

The observed average flux of the curvature radiation at the frequency ω is given by

$$\overline{F(\omega)} = \int_0^{2\pi/\Omega} F(\vartheta_K(t), \omega)\frac{\mathrm{d}t}{(2\pi/\Omega)} \qquad (2)$$

where ϑ_K versus t is

$$\cos\vartheta_K = \cos\vartheta_{\Omega B}\cos\vartheta_{\Omega K} + \sin\vartheta_{\Omega B}\sin\vartheta_{\Omega K}\sin\Omega t \qquad (3)$$

$\vartheta_{\Omega B}$ is the angle between the rotation axis and the magnetic axis, and $\vartheta_{\Omega K}$ is the angle between the rotation axis and the direction towards the observer.

By substituting into equation (2) the expression $F(\vartheta_K, \omega)$ given for the respective frequency intervals by (76)–(79) (ref. 11), we may obtain the integral flux $\overline{F(\omega)}$. In a partial case $\vartheta_K^{\min} \equiv |\vartheta_{\Omega B} - \vartheta_{\Omega K}| = 0$, when in the pulsar rotation process the centre of its curvature radiation beam may coincide with the direction towards the observer, the expression for $\overline{F(\omega)}$ is readily integrable.

The curvature radiation spectrum $\overline{F(\omega)}$ as a function of frequency dependence, for $\vartheta_{\Omega B} \sim 1$, $\vartheta_K^{\min} = 0$ and $\beta > 2$, is written as (see Fig. 1):

$$\overline{F(\omega)} \propto \begin{cases} \omega^{-(\beta-2)} & \text{at } \omega_B \ll \omega \leqslant \frac{9}{8}\frac{c}{R}\Gamma_0^3\hat{\vartheta}_0, \\ \omega^{-4(\beta-2)/(6+\beta)} & \text{at } \omega_m \ll \omega \ll \omega_B \\ \omega^{(6\beta-8)/(1+3\beta)} & \text{at } \omega \ll \omega_m, \end{cases} \qquad (4)$$

where

$$\omega_B = \left[\frac{3}{4}\left(\frac{2}{3}\right)^{2/3}\frac{(\beta-2)\,e^2\lambda^{\beta-2}\dot{N}}{mcR^2\Gamma_0}\left(\frac{8}{9}\frac{R}{c}\frac{1}{\Gamma_0^3}\right)^{-\beta}\right]^{1/(2+\beta)} \qquad (5)$$

$$\omega_m = \omega_B\hat{\vartheta}_0^{[16/3(2+\beta)]} \qquad (6)$$

where $\dot{N}$ is the number of particles ejected from a pulsar per unit time.

Both a break in the spectrum at ω_B and its low-frequency cutoff at the frequencies below ω_m result from the curvature radiation reabsorption by radiating particles as such. In the case considered, two characteristic frequencies appear in the radiation spectrum due to the non-uniformity and anisotropy of a curvature radiation source.

In the case of radio emission from pulsars, particles should be regarded as bunches of electron–positron plasma. Then in equations (5) and (6) the charge Q, mass M and the flux of

Table 1 Values of curvature radiation flux as a function of frequency dependence

	$\frac{6\beta-8}{1+3\beta}$	$\frac{4(\beta-2)}{6+\beta}$	$(\beta-2)$	ω_m (MHz)	ω_B (GHz)
$\beta=3$	−1	0.44	1	75	10
$\beta=4$	−1.23	0.8	2	134	8
$\beta=5$	−1.37	1.09	3	197	6.5

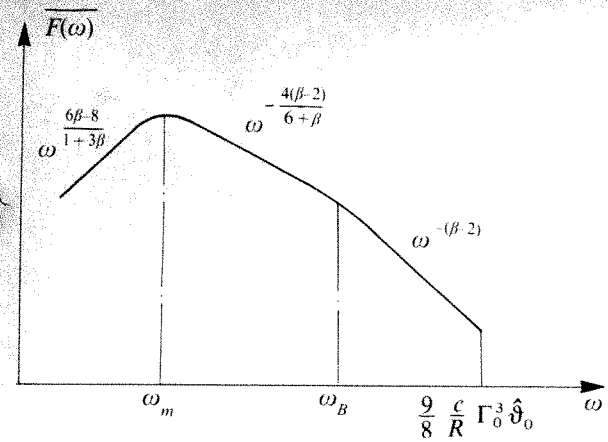


Fig. 1 The curvature radiation spectrum for $\vartheta_{\Omega B}\sim 1$, $\vartheta_K^{\min}=0$ and $\beta>2$.

bunches $\dot{N}_B$ from a pulsar should be used, rather than e, m and $\dot{N}$. The quantities are related as $Q=|n_+-n_-|e=\zeta ne$, $M=nm$ and $\dot{N}_B=\dot{N}/n$, where n_+ and n_- are the number of positrons and electrons in a bunch, respectively; $n=n_++n_-$, $\zeta=|n_+-n_-|/n$ is the degree of charge separation in the bunches of ejected plasma. Substituting Q, M and $\dot{N}_B$ in equations (5) and (6) shows clearly that ω_m and ω_B do not depend on n; there is, however, a relatively weak dependence of ω_m and ω_B on $\zeta(\omega_m, \omega_B\propto\zeta^{2/(2+\beta)})$. If the number of particles n in a bunch is changing, the radiation spectrum (see Fig. 1) shifts along the $\overline{F(\omega)}$ axis ($\overline{F(\omega)}\propto n$ at $\zeta=$constant), although it does not change its shape.

Table 1 summarizes the α values of the curvature radiation flux as a function of frequency dependence ($\overline{F(\omega)}\propto\omega^{-\alpha}$) for different frequency ranges, with β equal to 3, 4 or 5; ω_m and ω_B frequency values are also given for most probable parameters of pulsars[8-10,12]: $\dot{N}=10^{33}$ particles$\times$s^{-1}, $R=1.4\times10^6$ cm, $\Gamma_0=800$, $\hat{\vartheta}_0=10^{-2}$ and with ζ assumed equal to 0.1. In this case, whatever, the value of β, the radiation spectrum extends to the frequency $(9/8)(c/R)\Gamma_0^3\hat{\vartheta}_0$, which is $\sim1.7\times10^{11}$ Hz.

The following are the main observational data about low-frequency cutoffs in the pulsar radio emission spectra (for details see ref. 7). (1) The mean frequency of the pulsar radio emission maximum below which the radio emission flux decreases ($\alpha<0$), is $\omega_m=130\pm80$ MHz. (2) The mean value of the radio emission spectral index in the frequency range below the maximum ($\omega<\omega_m$) is -1.4 ± 0.4. (3) The spectral indices of pulsar radio emission at frequencies higher or lower than the maximum frequency are almost equal in absolute magnitude and opposite in sign, that is, the steeper the radio emission spectrum up to its maximum ($\omega<\omega_m$), the steeper it is after it ($\omega>\omega_m$).

Table 1 shows that when $\beta=4\pm1$ there is a good agreement between the expected behaviour of the curvature radiation spectrum, near its maximum, and the observational data. The ω_m versus β dependence is rather weak.

Above we have considered the case $\vartheta_K^{\min}=0$, when in the pulsar rotation process its magnetic axis may coincide with the direction towards the observer. With the results from ref. 11 it is easily shown that the ω_m frequency value and the curvature radiation spectrum behaviour near the maximum at $\vartheta_K^{\min}\neq0$ do not differ essentially from the case of $\vartheta_K^{\min}=0$ if

$$\vartheta_K^{\min}<\vartheta_r\equiv\frac{8}{9}\frac{R}{c}\frac{\omega_m}{\Gamma_0^3}\hat{\vartheta}_0^{-2} \qquad (7)$$

At $\omega_m=130$ MHz, $R=1.4\times10^6$ cm, $\Gamma_0=800$ and $\hat{\vartheta}_0=10^{-2}$, we have $\vartheta_r\simeq0.1$, that is, the ϑ_r value is sufficiently large ($\vartheta_r\gg\hat{\vartheta}_0$); hence for most pulsars $\vartheta_K^{\min}=0$ may be assumed when the curvature radiation spectrum is considered to be near its maximum.

Note that the model presented here for the generation of pulsar radio emission due to the curvature mechanism may also explain the radio emission spectrum behaviour at higher frequencies (see Fig. 1). Thus, the ω_B frequency (several GHz, see Table 1) falls into the interval of the observed values of the higher-frequency break of the pulsar radio emission spectrum[1,7]. The observed values of the spectral indices after the high-frequency break are ~2 to 3 (refs 1, 7, 8), which corresponds to $(\beta-2)$ for the same values $\beta\simeq4$–5.

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Structure, stability and evolution of Saturn's rings

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Recent data obtained from the Voyager spacecrafts and ground-based measurements indicate: (1) the rings have a thickness of at most 150 m (ref. 1) and probably several times less[2,3]; (2) the rings are mostly composed of ice particles ranging from centimetres to metres in size[4]; (3) the rings are subdivided into a large number of ringlets with a radial dimension ranging from 10-km down to the several metres resolution of the Voyager spacecraft's camera[5]; (4) the B ring contains very many optical depth variations (0.6–3)[3]. This behaviour is essentially determined by the collisional properties of the rings' ice particles. Here we report some preliminary results from an experiment designed to measure the coefficient of restitution of ice particles colliding at impact velocities relevant to Saturn's rings. We apply these results to simple dynamical models for Saturn's rings and deduce the rings' thickness to be <5 m. We also show that regions with optical depth <0.5, such as the B ring, are unstable to viscous diffusion. Such an instability may be the cause of optical depth variations in the B ring.

The global properties such as the ring thickness and dynamical evolution time scale are basically determined by the collisional dynamics between the ring constituents and the resultant transport processes[6–9]. The cumulative effect of many collisions over a differentially rotating disk, such as Saturn's rings, is to establish a viscous stress which induces an outward transfer of angular momentum, an inward diffusion of mass[10], and an energy transfer from systematic shear into dispersive motion at a rate $\dot{E}_i\approx v\Sigma\Omega^2r^2$, where Ω and Σ are respectively the angular frequency and surface mass density of the disk at radius r. The kinematic viscosity of the disk, is defined by $v\approx\sigma^2/[\Omega(\tau+\tau^{-1})]$ where $\tau=\Sigma A/m$ is the optical depth, A is the cross-section, m the mass of a typical ring particle, and σ is the average amplitude of the velocity dispersion. The particle collisions are partially inelastic and dissipate the kinetic energy associated with dispersive motion at a rate $\dot{E}_d\approx(1-\varepsilon^2)r^2\omega_c\Sigma\sigma^2$ where ε is the coefficient of restitution. The collision frequency, ω_c, is related to Ω such that $\omega_c=\tau\Omega$. A local energy equilibrium is established when $\dot{E}_i=\dot{E}_d$, that is,

$$(1-\varepsilon^2)(1+\tau^2)\approx b \qquad (1)$$

where b is of the order of unity. Assuming that the rings are

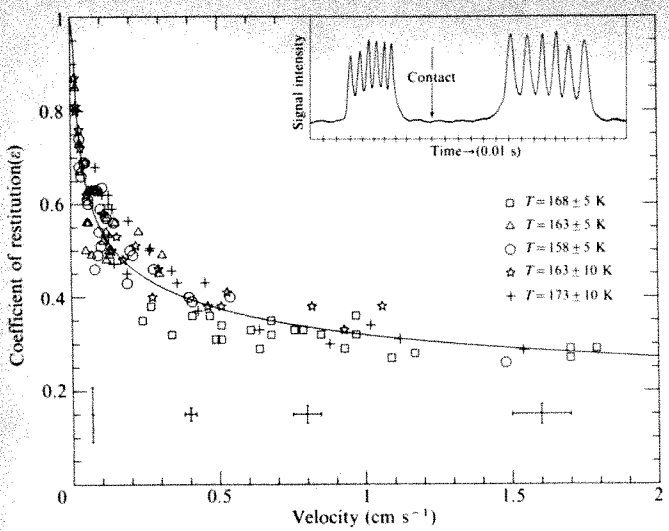


Fig. 1 A plot of the coefficient of restitution as a function of impact velocity for measurements at several different temperatures over the range 158–173 K. Estimates of the typical error bars are shown for four velocities at the bottom. The inset shows two sets of pulses obtained immediately before and after a collision. The values of ε and the velocity are obtained from the spacings of these pulses.

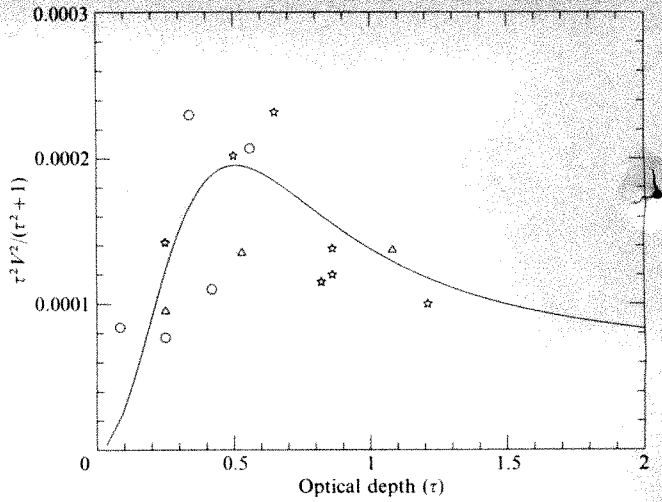


Fig. 2 A plot of $\tau^2V^2/(\tau^2+1)$ as a function of optical depth τ. The values for τ were obtained using equation (1). The points are obtained from the data in Fig. 1 and the solid line is a graph of $\tau^2V^2/(\tau^2+1)$ versus τ according to equation (4).

made of non-rotating, indestructable, and identical size ice spheres with a gaussian phase space distribution, b is determined from the rigorous treatment of the collisional Boltzmann equation[8]. An approximate analytical fit of these results indicates $b \approx 0.61$.

For most solid substances, ε is a function of the impact velocity, V (ref. 11). If an ε–V (or equivalently ε–σ) relationship is available, it can be applied to equation (1) to deduce a τ–σ relationship. If a disk is made up of nearly equally sized particles, its r.m.s. thickness, H, would be $\sim 2f\sigma/\Omega$. Here f is introduced to account for the anisotropy of the velocity dispersion ellipsoid and is of the order of unity ($f \to 1/\sqrt{6}$ as $\tau \to \infty$)[8,12]. Thus, an accurately determined ε–V relationship can be used to deduce H directly.

Another reason for determining an ε–V relation is associated with the viscous stability of disk flow. Several authors[13–15] have suggested that the condition for thermal and viscous stability cannot be satisfied simultaneously if $\tau \gg 1$. In these conditions the rings have a tendency to break up into many ringlets. This mechanism seems to offer a viable explanation for the origin of the density variations in the optically thick B ring where tidal effects due to various saturnian satellites are negligibly small. Physically, if the viscous couple, $W = 3\pi\Omega r^2 \Sigma \nu$, increases with τ, the rings are stable whereas if W decreases with τ the rings are unstable. The W–τ relationship can also be derived from an ε–V relationship. We have designed an experiment and determined this ε–V relationship in a laboratory environment.

To measure ε for ice particles in the conditions assumed to exist in Saturn's rings, the apparatus must meet several criteria. First, the relevant impact velocity must be in the range 0.01–5 cm s^{-1}. Second, the frictional damping of the system must be small compared with the kinetic energy associated with ice particle collisions such that the damping correction factor is relatively small. Third, the relevant ambient temperature should be in the range 100–150 K. Fourth, before and after each collision, the ice particles must be separated by a sufficiently large distance, say >0.01 cm, such that tiny powdered ice chips on their surface do not induce strong interactions between them except during the course of each collision. Finally the collisions should resemble free collisions as closely as possible.

The simplest experimental system which can satisfy most of the above requirements is to attach an ice ball to an oscillator and let it strike a stationary block of ice. A sufficiently low impact velocity and a reasonably large oscillator amplitude can be achieved with a compound pendulum with the axis of rotation very close to the centre of mass. In this case, the angular frequency is $(Mgd/I)^{1/2}$ where M and I are the mass and moment of inertia of the system. We constructed such a system using a disk mounted on an aluminium rod. Small weights at the top and bottom of the disk allow us to adjust the distance between the centre of mass and the suspension (that is rotation) axis, d. An ice ball is attached to one edge of the disk and a counter weight on the other edge in a manner such that their centre of mass is at the centre of the disk. The entire system is suspended by knife blades resting on polished agate plates. With this system, we have achieved periods as long as 60 s with the Q-value equal to 4. For shorter oscillation periods, such as $T < 30$ s, $10 < Q < 30$.

The main limitation of the above system is that collisions are not entirely free. The coupling between the ice particles and the pendulum body will cause a slight elastic deformation in the pendulum during a collision. However, we note that most of the kinetic energy dissipation occurs as a result of fracturing at the tiny contact surface area of the ice particles. For typical impact velocities the time interval during contact is considerably longer than the sound crossing time across the ice balls. Our experiments indicate that a sufficiently rigid mounting of the ice particle onto the pendulum effectively eliminated temporal redistribution of elastic energy within the system and thereby provided a good measure of ε.

The ambient temperature of the system is set at 150–175 K using a simple cryostat constructed from styrofoam and cooled with liquid nitrogen. The ice-block temperature is measured with an Omega thermocouple thermometer frozen into the ice block. The velocity of the ice ball is measured by reflecting a laser beam off a mirror mounted on the rotation axis of the pendulum. The reflected beam passes over a photocell masked with five slits 1 mm wide, 2 mm apart and situated 4 m from the pendulum. The photocell is positioned close to the point which corresponds to contact. Five pulses produced immediately before and after each collision provide an accurate measurement of the incoming and outgoing velocities from which ε can be deduced. Five slits are used primarily to improve the accuracy of the measurement and to eliminate damping contributions associated with the finite Q value of the pendulum. The results of our experiments indicate that only a small, that is <2%, correction is ever needed to account for damping.

A typical pulse train and the summary of the results obtained from the four lowest temperature runs are illustrated in Fig. 1. The general behaviour of ε as a function V is typical of the data taken at other temperature ranges. The data can be qualitatively divided into three cases. At relatively large V, ε changes little over a wide range of impact velocity. Below $V \approx 0.25$ cm^{-1},

ε increases rapidly with decreasing V. The extrapolation of our data indicates that, at a sufficiently small but finite velocity, ε approaches unity. This behaviour at the low velocity limit is consistent with the classical theory of nearly elastic deformation associated with low-velocity impacts[16–18]. However, the asymptotic plateau for relatively large velocity cannot be accounted for by any existing collision model.

The data presented in Fig. 1 can be fitted remarkably well with a power law

$$\varepsilon = (0.32 \pm 0.02)\, V^{-0.234 \pm 0.008} \quad (2)$$

where V is the impact velocity measured in cm s^{-1} for the range $0.015 < V < 5.1$. Extrapolation of this power law indicates that $\varepsilon \to 1$ for $V < V_c = 0.008$ cm s^{-1}. The dispersion of the data, relative to the best fitted curve, is much larger for intermediate collision velocities than for large velocities, where ε attains an asymptotic value of 0.25, and for very small velocities where ε approaches unity. Special care has been taken to eliminate various possible external causes for this large dispersion. A trial experiment for collision between a solid synthetic rubber ball (super ball) and a rigid surface yielded an ε–V relationship with dispersion of order less than one-tenth those found for ice ball-block collisions. Clearly, the scattering of the data is considerably larger than the typical measurement uncertainty which is indicated in Fig. 1. We propose that the large dispersion in the data is real, and is probably associated with compression and random powdering of tiny ice chips on the surface. Irregularities in the shape of the ice chips can lead to considerable variation in the radius of curvature and geometry of the contacting surfaces of the ice ball and block. The tendency for the dispersion to decrease at relatively large impact velocities is probably caused by the surface distortion during a collision being large enough to override any variation in the surface geometry immediately before each collision.

Note that the effective mass involved in a collision is 5.4 times larger than the mass of the ice ball mounted to the pendulum. Because the ice block is held stationary, it has an infinite inertial mass. In any event, as Saturn's rings are composed of ice particles with a large range in size, it is desirable to investigate collisions between particles with various masses and sizes. These investigations are underway and the results will be presented elsewhere.

Despite recent considerable observational and theoretical advances in our understanding of Saturn's rings, the actual physical nature of the constituent particles remains unclear. The rings may either be made of solid particles with relatively smooth surface radius of curvature or particles covered with loose ice chips[19]. The amount of gas and dust inclusions in the ring particles is also unknown. In our experiments, both the ice ball and ice block are prepared with a smooth surface and are relatively free of gas or dust. (The amount of surface frost on the ice ball has also been minimized although this can never be eliminated.) Furthermore our data are obtained for direct collisions whereas grazing collisions may be far more common in Saturn's rings. Thus, it is not entirely clear to what extent the data obtained from the above experiment are relevant to the structure of Saturn's rings. Nonetheless, we apply our results to the standard uniform-size hard sphere model[8] for simplicity. Under this assumption, V_c defines a lower limit on the thickness of the disk which is $\sim V_c/\Omega = 50(V_c/0.01\ \text{cm s}^{-1})(10^{10}\ \text{cm}/r)^{3/2}$ cm. If we apply the results of equation (2) to equation (1), it follows that

$$H = 2f\sigma/\Omega = 80f[1 - b/(1+\tau^2)]^{-2.1}(r/10^{10}\ \text{cm})^{3/2}\ \text{cm} \quad (3)$$

Applying an idealized, uniform-particle sized model, we derive for most regions of the ring that $H < 5$ m, provided the particle size is smaller than H. This value is consistent with that deduced from microwave scattering experiments[2]. The slight difference between these values and those deduced from resonant damping analyses[3] may be due to the additional energy source associated with external perturbers.

In the absence of external stimulation, such as Saturn's satellite resonances, the energy equilibrium implies an effective viscosity

$$Nu \approx 0.3(r/10^{10}\ \text{cm})^{3/2}\frac{[1 - b/(1+\tau^2)]^{-4.2}}{(\tau + \tau^{-1})}\ \text{cm}^2\ \text{s}^{-1} \quad (4)$$

and a corresponding diffusion time $T_d \simeq r^2/\nu$, longer than the age of the Solar System. The corresponding viscous stress is plotted in Fig. 2. Superimposed on the actual data is a W–τ relationship derived from equation (2) such that

$$W \sim [1 - b/(1+\tau^2)]^{-4.2}\tau^2/(1+\tau^2) \quad (5)$$

The maximum value of W is attained at $\tau_c = (1-b)/(4.2b-1)$. The scatter of the original data (Fig. 1) is amplified in Fig. 2 by numerous mathematical transformations of the experimental data. The results in equation (5) imply that, for $\tau \gtrsim 0.5$, W decreases with τ and therefore the rings are unstable. For $\tau \lesssim 0.5$, the rings are stable. If we were to take the widely scattered data points, the critical τ would lie in the range 0.3–0.7. Thus, the optically thick regions of the disk are expected to be diffusionally unstable and would open up low density regions with τ of the order 0.5–0.7. The observed optical depth profile of the B ring indicates that the typical τ in the less opaque regions of the B ring is ~0.6 (ref. 3).

In the above discussion we have not considered the effect of particle size distribution. Note that the large, metre-size particles have radii comparable to or larger than the ring thickness, as derived from equation (3), such that they may form a 'monolayer'. For these particles the velocity dispersion is determined by the gradient of the rings' keplerian velocity across their diameter[7] so that their W monotonically increases with τ and their motion is viscously stable. Consequently, the small centimetre-size particles and the large metre-size particles in the high τ B ring may have rather different radial structure. Voyager's data indicate that the primary contribution to the τ variation in the B ring is from the small particles. The large particles have a relatively smooth radial distribution. Thus, a realistic model for the evolution of the B ring requires a detailed analysis of the collisionally induced transport process for a collection of particles with different sizes (G. Stewart, in preparation). We intend to supplement the experimental data presented here with data on collisions between ice balls with different radii of curvature at the contact point.

The above results may also be applied to study the actual damping process in resonant excited density waves[20,21], the structure of narrow rings and sharp edges as well as the satellite shepherding phenomenon[22]. As for our experiments, variations in ice ball's and ice block's surface geometry, surface crystal lattice structure, collisional grazing angles, and ambient temperature will be examined in future. In general, we believe that the results presented here are a first step towards understanding ice-particle collisional processes.

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Topographic EXAFS

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The EXAFS (or extended X-ray absorption fine structure) method of absorption-edge spectroscopy for the determination of crystal bond lengths and coordination number, especially in the first coordination shell, is in very widespread use in chemical, crystallographic, biological and to some extent metallurgical research. The high-resolution method of X-ray diffraction topography, which reveals crystal defects, is likewise widely used in studies on crystal growth, phase transformations, chemical decomposition and mechanical properties and domain studies: that is, in the area of 'microstructure' rather than 'crystal structure'. It is obvious that the techniques are complementary and that both could be required to solve a particular problem—structural changes induced by segregation at a surface or internal interface, for example. However, up to now, EXAFS data have been taken only as an average over large samples, thus losing all microstructural information, and topographic data have not provided structural, bonding or chemical information. We present here the first experimental method of combining these experiments, simultaneously on one specimen.

The method relies on the properties of synchrotron radiation (SR)[1], whose high intensity and continuous spectrum enables EXAFS spectra to be accumulated in reasonable times (some tens of minutes) and X-ray topographs to be taken in some seconds. An obvious way of combining the methods would be to take an X-ray topograph to locate areas of interest, place an aperture of diameter equal to the resolution required in this area and measure successive EXAFS spectra during a linear or raster scan of the area. This method would certainly provide the best spectral data, but a rapid calculation of available intensities shows that with most current SR sources the data collection times would be quite impractical—days or weeks.

A possible way of speeding up the spectral data collection is the use of white radiation incident on the sample, followed by a dispersing element (for example, bent crystal) and a film or linear detector; this has recently been achieved[2,3] and results in dramatic decreases in data collection times. The topographic data can then be collected in a separate exposure.

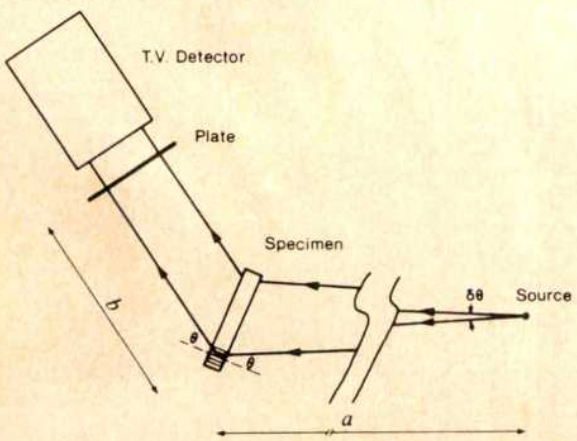


Fig. 1 Experimental arrangement for white beam topography. Typical specimen–source distances, *a*, are 20–80 m and specimen–film distances, *b*, 10–100 mm.

If a good quality single crystal is being used as the specimen, as it often is when topographic information is sought, a further and very simple technique is to use the specimen as its own monochromator. In the white radiation topographic method (Fig. 1), the incident beam diverges from the SR source, which is some tens of metres distant from the specimen. There is therefore a small variation of incident Bragg angle across a specimen, and a consequent variation of wavelength (energy) selected by the specimen for diffraction.

The experimental procedure is as follows. The orientation of the specimen is chosen and set so that the wavelength selected by some part of the specimen is at the absorption edge under

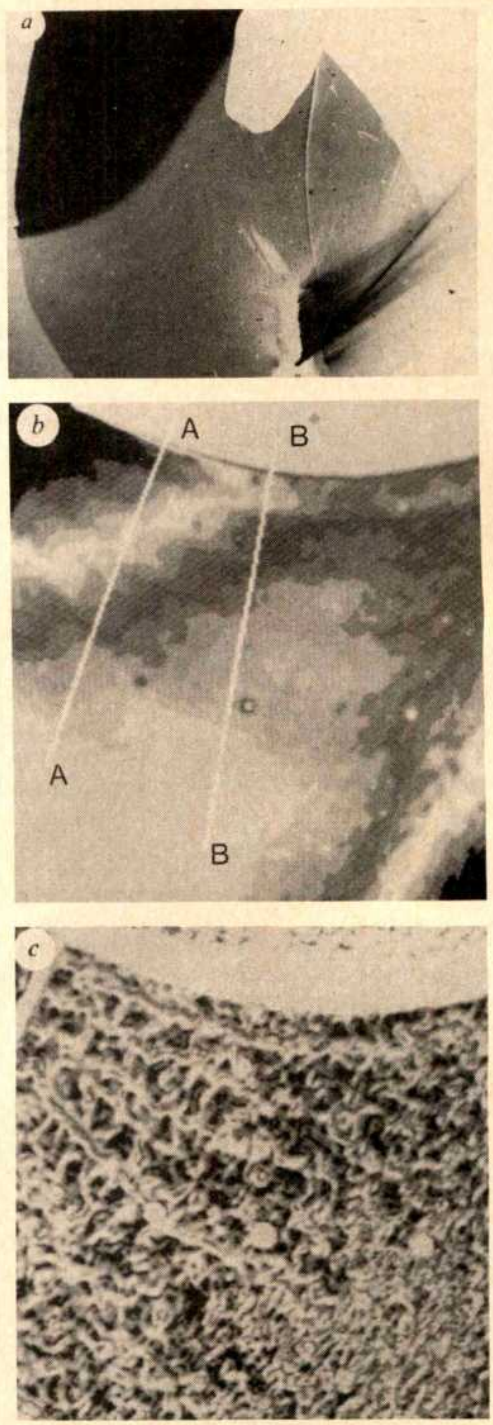


Fig. 2 *a*, White radiation topograph of Nb specimen (near perfect but with some elastic bending). 002 reflection, $\lambda = 0.65298$ Å at the absorption edge. SRS parameters 2 GeV 86 mA, 25 s exposure on Ilford L4 plate. *b*, Digitized and image processed (smoothed, threshold cut and contrast stretch) version of *a*. Image are 2.24 × 2.24 mm. *c*, Differentiated version of *b*.

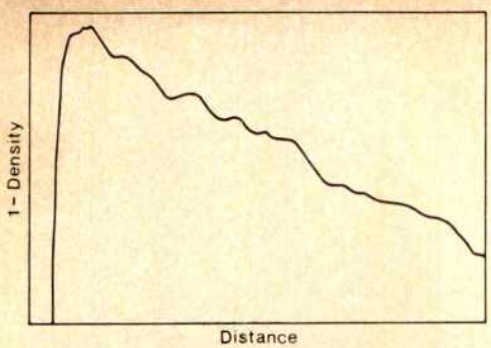


Fig. 3 Density traces extracted from Fig. 2, along line AA.

study. A standard topographic image is then recorded on high resolution plate, for example Ilford nuclear emulsion L4, exposed so that the high absorption side of the image is in good contrast. The wavelength selected by a point on the image is a direct function of the distance on the image from the edge, measured normally to the latter. The diffracted beam falling on each point will therefore have suffered scattering and absorption characteristic of a given photon energy. This results in 'fringes' in the image, parallel to the image of the edge, corresponding to maxima and minima in the EXAFS spectrum.

The first results, taken on the white radiation camera[4] at the SRS, Daresbury Laboratory, are shown in Fig. 2. Figure 2*a* shows the standard topographic image of a 125-μm thick Nb specimen at the Nb $K\alpha$ edge, reproduced photographically. The absorption edge is clear, but the fringes have weak contrast and are not evident. However, image processing techniques can be used to reveal them. The original plate was digitized on a Joyce Loebl flat bed densitometer with 5-μm resolution and the resulting matrix digitally smoothed and processed to cut off low and high values and to stretch the contrast. The result is shown in Fig. 2*b*; Fig. 2*c* shows a differentiated version of Fig. 2*b*. In each of these, especially the latter, the fringes are easily visible. Figure 2 shows density variations along the line marked AA in Fig. 2*b*. These show, albeit noisily, a typical EXAFS spectrum.

To demonstrate that these are indeed EXAFS-type data, comparison was made with an EXAFS spectrum taken on a rotating-anode generator of a 25-μm thick Nb foil. A rotating anode generator with a Mo target and a Si 800 bent crystal monochromator were used. The spectrum in Fig. 3 was calibrated for energy by comparison with the absorption edge positions in a set of topographs in which the specimen was rotated by 0.025° steps between each topograph. A composite photograph of the successive topographs is shown in Fig. 4. This provides an internal calibration that eliminates uncertainties due to curvature of the specimen, though it would not be necessary on a truly flat specimen. The energies corresponding to minima in the EXAFS spectrum are shown in Table 1 for the present results and the standard spectrum. The agreement leaves no doubt that this white-beam method measures true EXAFS data, simultaneously with the topographic image, with an exposure time of 25 s in this case.

Table 1 Comparison of X-ray energy at spectral minima in data extracted from the topograph (Fig. 2*b*) and from a standard EXAFS spectrum

Fringe edge	From topograph (keV)	From spectrum (keV)
	18.99	18.990
1	19.07	19.074
2	19.13	19.124
3	19.19	19.180
4	19.24	19.252
5	19.29	19.330

Estimated errors are ±0.01 keV from the topographic data and ±0.005 keV from the spectral data with the exception of fringe 5 (rather broad) where they are each about ±0.02. The topographic data were calibrated relative to the edge taken as 18.99 keV.

We need to know what is the quality of the EXAFS spectra obtained by this means (the images show normal topographic resolution and contrast). There are several factors to be considered: (1) dispersion and energy range; (2) effect of finite source height; (3) effect of source divergence; (4) wavelength acceptance of crystal reflection; (5) spatial resolution of photographic plate. Let the source height be h, the source–specimen distance a, the specimen–detector distance b, and the Bragg angle θ. ψ_0 and ψ_h are as usual the angles made by the incident and diffracted beams with the inward-facing surface normals and $\gamma_0 = \cos\psi_0$, $\gamma_h = \cos\psi_h$. Examples of the application of the equations will be given for the case corresponding to the

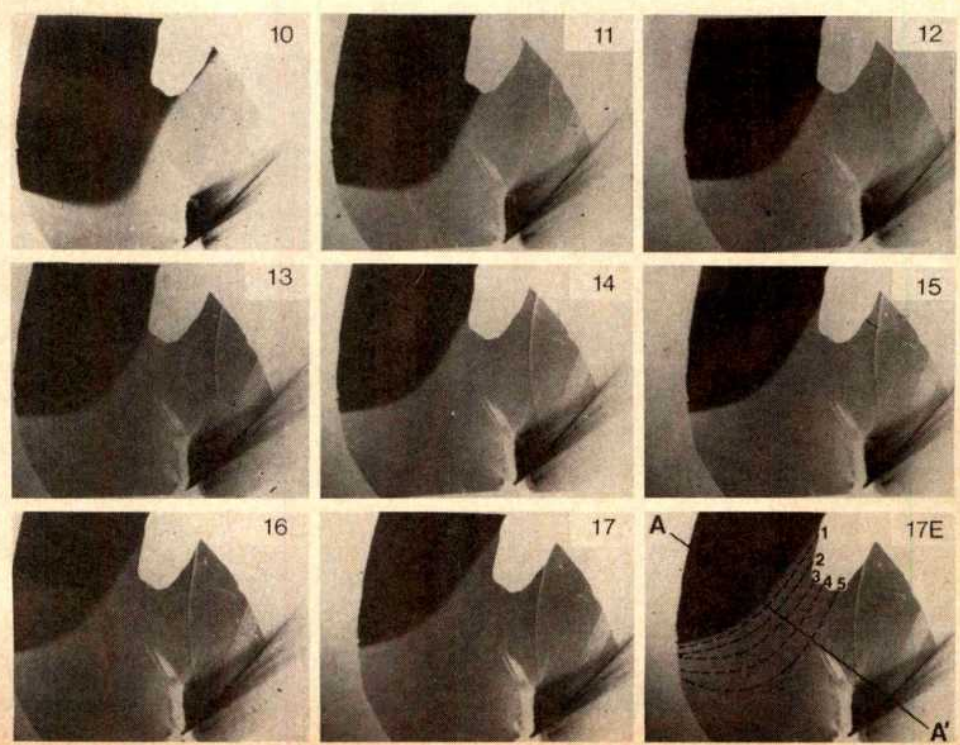


Fig. 4 Composite topograph of area shown in Fig. 2, showing successive positions of absorption edge as specimen rotated in 0.025° steps.

experiment reported above: Nb 002 reflection, $\gamma_0 = \gamma_h = 1$, d-spacing 1.6503 Å, $\theta = 11.4105°$ at the K absorption edge which is at 0.65298 Å, $a = 81$ m, $b = 75$ mm.

The position of a point on the image is defined by its perpendicular distance x to the absorption edge, with the photographic plate set normal to the diffracted beam. Straightforward geometry gives, assuming for the moment a point source, a dispersion relation:

$$\frac{\delta\lambda}{\lambda} = \frac{\gamma_0}{\gamma_h} \cot\theta \frac{x}{a} \quad (1)$$

if b is small, otherwise (because the diffracted rays converge from the specimen)

$$\frac{\delta\lambda}{\lambda} = \cot\theta \frac{x}{a\frac{\gamma_h}{\gamma_0} - b} \quad (2)$$

For the Nb example this gives a wavelength or energy dispersion of 3.06×10^{-7} per 5 μm digitizing element. Over a 10-mm image, the range is thus 6.12×10^{-4}. These figures correspond to 0.0058 eV resolution and 11.6 eV range. This spectral resolution cannot be achieved in practice for various reasons, discussed below, but the range in the Nb sample discussed above was ~300 eV over 2 mm (from internal calibration), therefore the specimen was curved with ~0.63 m radius of curvature. One purpose of recording the absorption edge position as a function of inclination was to determine this curvature, near a notch in the specimen. The dispersion range is thus adequate for XANES (X-ray absorption near-edge structure) measurements on a flat specimen, but for a full EXAFS spectrum several topographs should be taken at different inclinations. Most SR beam lines are much shorter than 80 m and so greater energy spread would occur.

The finite source height (in the incidence plane) has a great effect on the resolution. Imagine a parallel beam the height of the source falling on the specimen. This will be diffracted to a beam the same height (multiplied by the asymmetry factor γ_h/γ_0) and be recorded on the film, blurring spectral data in this region. The minimum useful area on the film is therefore $h\gamma_h/\gamma_0 = x_{min}$. This can be combined with the dispersion equation (1) to give the minimum spectral resolution

$$\frac{\delta\lambda}{\lambda_{min}} = \frac{h \cot\theta}{a} \quad (3)$$

(with a corresponding equation if equation (2) is used). For the niobium example with the SRS FWHM value of $h = 300$ μm, we obtain for the Nb example

$$\frac{\delta\lambda}{\lambda_{min}} = \frac{\delta E}{E_{min}} = 1.84 \times 10^{-5} \text{ or } 0.35 \text{ eV}$$

If now x is taken to be $h\ \gamma_0/\gamma_0$ then the apparent source divergence is $2h/a$. For the SRS at $a = 81$ m, this results in

$$\frac{\delta\lambda}{\lambda} = \cot\theta\ 7.4 \times 10^{-6} \quad (4)$$

For Nb_{002}, this becomes 3.7×10^{-5}, ~0.7 eV.

The effect of the wavelength acceptance of the crystal is found from the FWHM of the rocking curve as $\delta\theta$, using $\delta\lambda = \cot\theta\delta\theta$. For an appropriate FWHM ~5 arc s, this gives

$$\delta\lambda = 1.72 \times 10^{-4} \text{ or } 3.25 \text{ eV}$$

The effect of the spatial resolution of the plate ≲1 μm, is quite negligible on the spectral resolution ($\delta\lambda/\lambda \sim 10^{-7}$), because the intrinsic dispersion is so high.

Combining these three effects (source height, divergence and wavelength spread) by taking the root of the sum of the squares leads to an energy resolution in the Nb case of 1.3×10^{-4} or ~3.4 eV. This is perfectly acceptable for the purpose. The dominant factor is the wavelength acceptance of the crystal, and this could easily be decreased by using a weaker reflection.

The signal-to-noise ratio on the spectrum in Fig. 3 is poor and not normally acceptable for a useful EXAFS spectrum: note that the specimen was about five times thicker than the ideal. However, only 4 pixels were involved in each smoothing operation, giving 20 μm spatial resolution, whereas the minimum useful pixel size is $h\gamma_h/\gamma_0 = 300$ μm in this case. Because nuclear emulsion can record up to about 5 photons μm^{-2} at this wavelength, smoothing over a 300-μm range would give a dynamic range of $5 \times 300^2 = 4.5 \times 10^5$ and a corresponding precision of 0.15%. Whilst this is still not as good as the best EXAFS experiments it is acceptable for many purposes.

What then are the applications of this technique? Whilst it is a rapid and convenient way of obtaining EXAFS spectra if good crystals are available, often they are not, at least without considerable effort. The interest must lie in the ability to perform chemical and defect crystallography on the same sample, as can be performed (on very different samples) by the electron energy loss method[5]. Applications such as structural changes near interfaces with segregation or at decorated dislocations, valence state changes in defect ionic compounds (for example, iron salts, some of which are important catalysts), structural changes at the onset of chemical decomposition are possible. Whilst in principle one could scan the specimen in orientation to reconstruct the complete spectrum for each pixel on the image this would probably not be necessary in practice. A structural or chemical change at a pixel, from sufficient volume of the specimen, would result in a distortion of either the position or the intensity of the local fringe pattern. This would often provide the clue to anomalous behaviour, which could be followed up with techniques such as microbeam EXAFS over a small, closely defined area, or microbeam trace element analysis, all using the same apparatus.

It is also apparent that similar contrast is to be expected in radiographs taken near absorption edges with non-dispersive monochromators. With brighter and more intense sources, TV detectors and image processors, dynamic topographic EXAFS should be a real possibility.

We thank Professor A. J. Forty (University of Warwick) who first encouraged us to look for techniques of microbeam EXAFS, and Dr P. Georgopoulos (Northwestern University) for help in obtaining the reference Nb EXAFS spectrum, and Daresbury Laboratory staff for cooperation and the SRS facilities. The work was carried out with the support of the UK SERC (grant GR/B61283 and the US ONR (contract N00014-C-1012).

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Natural chlorine isotope variations

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Previously known natural variations in the stable isotope ratio of chlorine, $^{37}Cl/^{35}Cl$, have been within the experimental error of ±1.0‰. [All our measurements are reported in terms of δ^{37} in units of ‰; $\delta^{37}Cl = |(Rx/Rs) - 1| \times 1{,}000$, where $R = {}^{37}Cl/{}^{35}Cl$.] Using methyl chloride gas in a ratio mass spectrometer, we have increased the precision of measurement to ±0.24%, and report here significant isotope variation in natural chlorides. In general, salt deposits and saline hydrothermal springs tend to be enriched in ^{37}Cl with respect to seawater. Groundwater shows both enrichment and depletion with respect to seawater. Saline groundwater becomes more enriched in ^{37}Cl with increasing depth.

Eighty years of chlorine isotope studies[1-9] have shown that the stable isotope ratio, $^{37}Cl/^{35}Cl$, is remarkably constant in nature. Hoering and Parker[7] used HCl gas in a mass spectrometer to measure stable chlorine isotope ratios in 81 samples of natural materials; they found no significant variation beyond their stated limit of 1.0‰, although they did report two samples, both from a deep aquifer brine, as being possibly fractionated. Rankama[10] summarized the bulk of chlorine isotope studies when he wrote, "[Past work] suggests that additional accurate measurements of the isotopic constitution of chlorine in carefully selected samples are required to investigate the possible natural fractionation of the chlorine isotopes". We have used a technique of measurement which is more precise than those used in previous studies, and focused on comparing subsurface brines with seawater and other natural chlorides.

The measurements reported here were made by mass spectrometry of methyl chloride ions (CH_3Cl^+), using the method of Taylor and Grimsrud[11] to prepare methyl chloride gas. Chlorine is quantitatively precipitated as silver chloride (AgCl) from aqueous solutions with silver nitrate. AgCl is then reacted with methyl iodide for 2 days in a reaction vessel at 70–100 °C. The product, CH_3Cl, is then separated from excess methyl iodide by gas chromatography and yields measured to ensure that all of the chlorine is recovered. An isotope ratio mass spectrometer (V. G. Micromass 602C) compared sample CH_3Cl with a commercially-produced reference gas to a precision of ±0.03‰. The total analytical standard deviation, determined from more than 20 replicates of our laboratory standard NaCl over a 1-yr period, is ±0.24‰. Following Taylor and Grimsrud's example, this standard deviation of 0.24‰ is used as the precision for the measurement technique. The mass spectrometer collector slit width masks all but the $m/e = 52$ peak for the minor beam and the $m/e = 50 + 51$ for the major beam. Variations of $^{13}C/^{12}C$ and D/H are minimized by using the same batch of methyl iodide and repeated standardization. Fragmentation patterns should be the same for equally pure sample and reference methyl chloride on a given day's run, and in practice seem not to vary significantly even with filament changes. Frequent monitoring of the constancy of the $51/(49 + 50)$ difference between reference and sample ensures the validity of the data.

Repeated measurements of eight oceanic chloride samples, one from the Gulf of Mexico off the coast of Texas and the others from depths up to 600 m in the Pacific Ocean, gave a total spread of 0.12‰ and a standard deviation of 0.05‰. Results reported here are relative to this standard mean oceanic chloride (SMOC) isotope ratio ($^{37}Cl/^{35}Cl_{oceans} \simeq 0.324$), thus the average $\delta^{37}Cl$ for ocean water is set at 0.00‰.

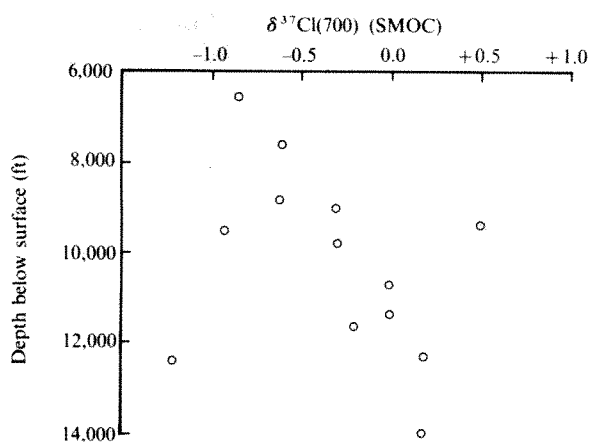


Fig. 1 Variation of $\delta^{37}Cl$ with depth of sample collection in oil field brines from east Texas and Louisiana. The Pearson product moment correlation coefficient for these data is +0.61 and is significant to within the 95% confidence limit[14,15]. Excursions from the apparent linear trend may represent vertical mixing of brines through geological heterogeneities and cracks in well casings. The correlation of ^{37}Cl enrichment with depth may reflect an isotope fractionation mechanism related to the electrostatic properties of subsurface clays (ref. 13 and F. Phillips and H.B., in preparation).

Table 1 shows the results of several groups of samples that we have analysed. Chlorine isotope ratios of aquifer brines from east Texas and Louisiana, including those shown in Table 1, are plotted in Fig. 1 as a function of approximate depth of sample collection.

Table 1 indicates that significant chlorine variation in natural environments does occur, although most of the variation is within 1.0‰ of ocean water chlorine. The range of variation shown is ~2.1‰ with a precision of 0.24‰. Although little can be said about the variations in terms of mechanisms of fractionation, the measurements indicate some distinct geochemical tendencies. Salt deposits tend to be enriched in ^{37}Cl with respect to seawater, as do hydrothermal samples. Groundwater samples may be either enriched or depleted in ^{37}Cl with respect to seawater. The two shallow groundwater samples shown in Table 1 are from the same aquifer, suggesting that within one aquifer there may be more than one chloride source and/or isotope fractionation mechanism. In brines from deep aquifers, increasing isotope enrichment seems to correlate with increasing depth (Fig. 1). Total chloride concentration of these brines also correlates with isotope composition within each aquifer.

To date, all of the samples measured in our study contain chlorine as chloride ions which limits the likely mechanisms for enriching isotopes to precipitation and diffusion processes. $Na^{37}Cl$ may precipitate more readily than $Na^{35}Cl$ just as $^{34}SO_4$ precipitates more readily than $^{32}SO_4$ during gypsum formation[12]. Measurements on NaCl deposits in Table 1 may reflect isotope fractionation during formation. Counter-current electromigration, a laboratory method used to separate chlorine isotopes[13], may be analogous to chlorine isotope fractionation in groundwater (F. Phillips and H. B., in preparation). During counter-current electromigration experiments, chloride in a NaCl solution is driven through a sand-packed tube by an electrostatic force against the convective movement of the solution. ^{35}Cl

Table 1 Results of analyses

	$\delta^{37}Cl$ (‰)*	Student's *t*-test results (probability of variance from SMOC[14,15])
Ocean water samples		
(14 measurements on eight samples, s.d. 0.05‰)	0.00	—
Halite samples		
Bedded Salt, Salina Formation, Ohio (mean of 21 measurements, s.d. 0.24‰)	+0.52	>0.99
Weeks Island Salt Dome, Louisiana (mean of four measurements with total spread of 0.08‰)	+0.20	0.98
Hydrothermal samples		
Morgan Hot Spring, California	+0.40	0.92
Crater Lake, New Zealand	+0.45	0.98
El Chichon crater water, Mexico	+0.41	0.95
Shallow groundwater samples (<150 m)		
Milk River aquifer sample no. 17, Canada	+0.8	0.98
Milk River aquifer sample no. 1, Canada	−0.4	0.96
Deep groundwater samples (>2,000 m)		
WC-W155, Louisiana	−0.36	0.85
81-1085, Texas	−0.62	>0.99
82-090, Texas	−0.95	0.95
81-1082, Texas	+0.53	>0.99
81-1124, Texas	−1.29	>0.99

* All data reported with respect to SMOC.

diffuses more effectively than ^{37}Cl towards the cathode end of the tube. In a groundwater analogue of counter-current electromigration, the electrostatic force may be provided by charged clay mineral surfaces and streaming potential produced by forced convection of water. The convective movement is induced by natural hydraulic and osmotic pressure gradients. Although discussions of fractionation mechanisms based on our results are speculative, experiments and the construction of theoretical models designed to clarify the nature of possible mechanisms are underway.

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Suboxic diagenesis in banded iron formations

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Anomalous isotopic composition has been reported for the carbon in carbonate minerals of banded iron formations. Well studied examples show an enrichment in the light isotope of carbon, ^{12}C. This enrichment presumably reflects unusual circumstances in the deposition of these sedimentary rocks. It is suggested here that the isotopically-light carbonate results from early diagenetic oxidation by bacteria of substantial amounts of isotopically light organic carbon. The electron acceptor that permits oxidation in the absence of free oxygen is presumed to be iron(III) which may have been significantly more abundant in the initial chemical precipitate than in the post-diagenetic sedimentary rock.

Banded iron formations are chemical sediments, formed by precipitation of dissolved species from solution[1]. They are composed principally of cryptocrystalline or crystalline quartz, iron oxides (haematite and magnetite), iron silicates (greenalite and related minerals) and iron carbonates (siderite and ankerite)[2]. Banded iron formations are major sources of iron ore. Essentially all of them were deposited before $\sim 1.7 \times 10^9$ yr ago[3], an observation that has been interpreted to reflect a transition at about this time from a hydrosphere devoid of oxygen to a hydrosphere rich in oxygen[4,5]. Because oxidized iron(III) is almost insoluble in all but very acid solutions, the transport of iron in solution and its subsequent chemical precipitation in banded iron formations must have occurred in anoxic waters. The extensive area of some banded iron formations and substantial rates of accumulation of iron and silica seem to imply deposition in basins that were open to the ocean[6–9].

The isotopic composition of the carbonate minerals in banded iron formations has been studied most thoroughly in formations of the Hamersley Basin of Western Australia[9,10]. These undeformed sedimentary rocks have been subjected to only low-grade metamorphism[11]; they date from $\sim 2.5 \times 10^9$ yr ago[12] and appear to have been deposited on a submarine platform or bank protruding into or marginal to an ocean[9]. Carbonate minerals are fairly abundant, typically making up between one-tenth and one-half of the rock[10,13]. Becker and Clayton[14] studied the isotopic composition of the carbonate minerals in the Dales Gorge Member of the Brockman iron formation and reported an enrichment in the light isotope of carbon. Expressed as the departure of the ratio of carbon isotopes from the ratio in a standard carbonate, this departure amounted to an average of $\sim 10‰$, expressed as $\delta^{13}C = -10‰$. This finding has been confirmed and extended by Baur *et al.*[10], who studied the underlying Marra Mamba and Mt Sylvia formations as well as the Dales Gorge Member of the Brockman iron formation. These authors found variable isotopic compositions, but in all cases the carbonate minerals were strongly enriched in the light isotope of carbon. Similar results for banded iron formations in North America have been reported[15,16].

Little isotopic fractionation with respect to dissolved carbonate results from the precipitation of carbonate minerals including the iron carbonates, siderite and ankerite[17], so most marine carbonates have isotopic compositions close to the standard, $\delta^{13}C \sim 0‰$. Indeed, isotope measurements on marine carbonate rocks of different ages are used to infer the history of the carbon isotopic composition of seawater. Such studies have revealed only minor variation of seawater carbon from a value near $\delta^{13}C = 0‰$ since at least 3.5×10^9 yr ago[18–20]. There is no reason to suppose that the isotopic composition of the ocean was any different at the time of deposition of the banded iron formations of the Hamersley Basin. Indeed, the Wittenoom Dolomite[10,14], a non-iron formation carbonate rock immediately overlying the Marra Mamba iron formation, has a carbon isotopic composition of $\delta^{13}C = 0.36‰$ very close to the marine carbonate standard. It does seem likely, therefore, that the carbonate minerals in the iron formations were deposited with isotopic compositions different from that of the seawater from which these minerals were ultimately derived.

Isotopically-light marine carbonate in Phanerozoic sedimentary rocks would generally be attributed to the oxidation of reduced organic carbon during early diagenesis[21]. Reduced organic carbon is strongly enriched in the light isotope, $\delta^{13}C = -25‰$ or lower[22,23]. Diagenetic oxidation of organic carbon in young sediments can dilute the seawater carbonate dissolved in the pore waters of the sediments, yielding isotopically light dissolved carbonate[24]. The carbonate minerals that equilibrate with these pore waters can also be isotopically light. Data on isotopically light marine carbonates of Mesozoic age, presumably produced in this way, have been presented by Irwin *et al.*[21]. Could this process not also have been responsible for the isotopically light carbonates in banded iron formations, a possibility suggested by Baur *et al.*[10]?

The problem with this mechanism is to identify the electron acceptor that made possible the oxidation of organic carbon in iron formations. The assemblage of minerals in these formations provides clear evidence for a very low oxygen fugacity in the pore waters of the sediment[2]. This conclusion is not contradicted by the presence of some iron(III), which is stable even at low oxygen fugacities. So the electron acceptor was not oxygen. There is also no reason to suppose that dissolved nitrate was abundant on the anoxic Earth[25]. But the oxidation process evidently did not involve the reduction of dissolved sulphate to sulphide, because sulphide minerals are not abundant in the iron formations of the Hamersley Basin[3]. Data[26] on the Dales Gorge Member of the Brockman iron formation reveal maximum concentrations of ~ 0.3 wt% and average concentrations much lower. Iron sulphides are relatively insoluble, and any sulphide produced in the sediments by diagenesis would have been precipitated by reaction with the abundant dissolved iron[27,28]. The apparently minor role of sulphate reduction may have been a consequence of low sulphate concentrations in the

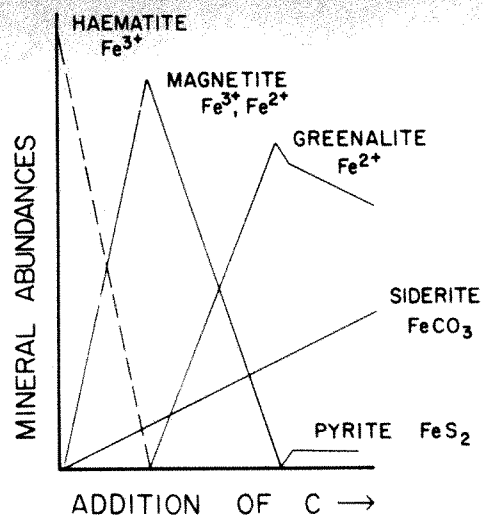


Fig. 1 Suboxic diagenesis of increasing amounts of reduced organic carbon causes the progressive reduction of iron(III) to iron(II). The result is that haematite is replaced by magnetite which is in turn replaced by greenalite and related silicate minerals that contain little or no iron(III). Sulphate reduction and the precipitation of pyrite do not commence until all iron(III) has been reduced. The carbonate produced by diagenetic oxidation of organic carbon precipitates as iron carbonate minerals, siderite and ankerite. The oxide, silicate, and sulphide facies of banded iron formations may reflect increasing amounts of organic carbon in the original sediments.

Archaean and early Proterozoic ocean[29–31]. In recent sediments, when sulphate is exhausted, further diagenetic oxidation of organic matter uses organic matter as the electron acceptor. The metabolic processes are fermentation and methanogenesis. Methanogenesis, however, produces isotopically very light methane and isotopically heavy carbonate[24]. This process could not, therefore, be the source of the isotopically-light carbonates in the banded iron formations. (For a contrary opinion see ref. 10.)

It has recently become clear, however, that iron(III) can serve as electron acceptor in the early diagenetic, bacterial oxidation of organic carbon (see refs 28, 32–34). There is good evidence that particulate iron(III) is reduced to dissolved iron(II) during the anaerobic oxidation by bacteria of reduced organic carbon. The reduction of iron occurs after dissolved oxygen and nitrate have been exhausted and after the reduction of oxidized manganese, but before the reduction of sulphate[32]. Banded iron formations are, of course, rich in iron. Iron(III) could well have been the electron acceptor that permitted diagenetic oxidation of isotopically-light organic carbon by bacteria to produce the isotopically-light carbonate minerals in iron formations[10,15]. A stoichiometrically equivalent reaction was proposed by Perry *et al.* as an abiological, metamorphic process.

The initial oxidation of dissolved iron(II) to particulate iron(III) would have occurred in the water column, possibly near the surface. There are several oxidation processes that would not have required significant concentrations of free oxygen. Abiotic photochemical oxidation has been suggested and investigated experimentally[35,36]. Iron(II) may have served as electron donor in a metabolic process of bacterial photosynthesis[37]. Alternatively, the electron donor for biological photosynthesis may have been water, with the oxygen produced in the process consumed immediately by reaction with excess dissolved iron[4].

Consider an iron formation that contains 5 wt% of CO_2 in the form of carbonate minerals[26]. Suppose that this carbon has an isotopic composition of $\delta^{13}C = -10‰$, which represents a mixture of equal parts of oceanic carbonate with $\delta^{13}C = 0‰$ and diagenetic carbonate produced by the oxidation of organic matter with $\delta^{13}C = -20‰$. The oxidation of 0.7 g of organic carbon per 100 g of rock is required. This would not represent an unreasonably large concentration of organic carbon in the initial sediment.

Oxidation of this carbon according to the stoichiometric relationship

$$C + 2\,Fe_2O_3 \rightarrow CO_2 + 4\,FeO$$

would produce 16 g of FeO per 100 g of rock. Iron formations, including those of the Hamersley Basin, typically contain from 20 to 25 wt% of FeO by weight[3]. The proposed role for early diagenetic oxidation of reduced organic carbon by iron(III) is therefore not contradicted by data on the iron(II) content of banded iron formations.

On the other hand, if 0.7 g of organic carbon were oxidized according to the stoichiometric relationship

$$2C + H_2SO_4 \rightarrow 2CO_2 + H_2S$$

the yield would be 0.9 g S per 100 g of rock. The average sulphide concentration of banded iron formations, including those of the Hamersley Basin, is substantially less than this[3,26].

What are the implications of an early diagenetic source for much of the carbonate in banded iron formations, a source involving the oxidation of reduced organic carbon and the reduction of oxidized iron(III)? First, because this early diagenetic process is a consequence of the metabolic activities of bacteria, the isotopically light carbonate in banded iron formations may be evidence for the presence of bacteria in the sediments that became banded iron formations[38]. Microfossils have not yet been discovered in the banded iron formations of the Hamersley Basin[38]. The calculations presented above suggest that a large fraction of the total iron(II) in these formations may have been reduced by bacteria during early diagenesis. The initial deposits, in this view, contained iron largely in the iron(III) form together with reduced organic carbon in an amount that was stoichiometrically comparable. The preserved ratio of iron(III) to total iron in these sediments is therefore not primary, but is a product of early diagenesis. Also, the generally low concentration of organic carbon in banded iron formations[3] is explained as a consequence of the early diagenetic oxidation of an initially much larger amount of carbon by the oxidized iron of the formations themselves. Finally, the oxide, silicate, and sulphide facies of iron formations[2,39] may reflect a response of the sedimentary system to the early diagenetic alteration of progressively larger amounts of reduced organic carbon (Fig. 1). The oxide facies contains both iron(II) and (III), the silicate facies contains principally the iron(II) form, and the sulphide facies contains iron(II) and sulphide that is presumably the product of bacterial sulphate reduction.

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33,000-yr old chert mining site and related *Homo* in the Egyptian Nile Valley

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The Nazlet Khater 4 site (Nile Valley, Upper Egypt) is located on one of the small wadi-interfluves in the lower desert near the steep cliffs bordering the western Nile Valley edge (Fig. 1). We have previously reported[1] the excavation of an early Upper Palaeolithic blade industry at this site, although blade industries in the Nile Valley had only been known[2] to occur since 18,000 yr ago. The 1982 excavation reported here confirms that Nazlet Khater 4 is a chert mining site with a complex extraction strategy, going back 33,000 yr. A nearby grave contained a skeleton of a man in the extended position. We show that the cranial morphology is anatomically modern with archaic characteristics such as a very robust mandible. There is evidence that the skeleton has a similar age to that of the mining site.

The 1982 excavation enabled us to distinguish three sedimentary units: (1) the substratum of the site or the pre-Upper Palaeolithic natural deposits, (2) Upper Palaeolithic diggings in the substratum and their related infills, and (3) later deposits on the site.

The basement of the site consists of greenish silts and fine sands covered by a 1.5-m thick Nile channel deposit, composed of channel lag deposits of well-rounded Nile gravels and chert cobbles and of brown granuliferous silts, covered by a wadi deposit of local limestone gravels (Fig. 2).

Three Upper Palaeolithic digging activities can be distinguished (Figs 1, 2). (1) A trench, 9×2 m, with a depth of 1.5 m had been opened at the northern edge of the Nile deposit, just beyond the covering wadi deposit. All the Nile deposits had been removed from this trench which represents $\sim 15\ m^3$ of ground. (2) Vertical shafts, dug down to the channel lag gravel, had sometimes been enlarged at their base to form bell pits. (3) From the walls of the trench or from the bell pits, the channel lag gravels had been extracted from the substratum for several metres, creating short galleries.

The deposits in the trench and in the bell pits consist of two lithological units: gravels at the base, covered by yellow fine aeolian sand. The gravel unit is a purely gravitational deposit, composed mainly of Nile pebbles, mixed with coarse sands, silts, local limestones, Upper Palaeolithic artefacts, and a fragment of gazelle horn core. Several hearths *in situ* with an abundance of charcoal were observed. This gravel unit is heavily encrusted by a 10-cm thick calcrete on its surface. The top of this unit forms a depression and represents an occupation floor with a large hearth.

The overlying infilling of loose aeolian sands completely hides all Upper Palaeolithic diggings. In the trench, three very slightly encrusted layers with gravels and derived artefacts are intercalated[1]. During the formation of this unit, important deflation and sand accumulation, not found in the gravel layers, took place. The presence of an *in situ* hearth in this sand unit indicates that the site was occupied during this stage. The post-occupation evolution of the site consists of the formation of a salt crust with desiccation wedges and in the flattening out and re-installation of the overall perfect gently eastward dipping slope.

We have currently nine ^{14}C dates at our disposal (Fig. 2). Samples GrN-11297, GrN-11300, Lv-1139D, GrN-11301 and GrN-11299 are from different hearths; samples Lv-1142D, Lv-1141D, Lv-1140 and GrN-11296 are from dispersed charcoal. All dates, ranging from 35,100 to 30,360 yr BP, confirm the great age of the mining site. Statistical tests on different combinations of these samples indicate that they do not belong to the same population (significance level better than 99%), so that it is inappropriate to calculate a mean age for the site. It seems justifiable, however, to classify the ^{14}C dates from the trench according to their lithological positions. The weighted average for the three samples in the gravel unit is $33,470 \pm 360$ yr BP, and for the three samples in the sand unit, $31,980 \pm 650$ yr BP. As their significance level is 5%, we suggest that the mining activities were spread over a period of time which can be determined by ^{14}C dating.

Large quantities of lithic artefacts were recovered. The raw materials are the chert cobbles from the Nile channel lag deposits. Flat elongated chert cobbles were chosen. Flaking of short but regular blades was performed by direct blows with a soft hammer. The butts are flat or punctiform, the bulbs are thin; the Levallois technique was not used.

Tools are rare and are made from flakes or blades by flat retouching, sometimes bifacial, or by oblique retouching. Denticulates aré the best represented tools although some burins and end-scrapers are present. Some bifacial axes have been collected; these are flat, made from flakes or blocks, with concave sides and a convex cutting edge (Fig. 3*a*, *b*).

The Upper Palaeolithic trenches in the substratum were clearly intended for the extraction of chert cobbles which were subsequently used for flaking. The method of exploitation consisted of undercutting the brown granuliferous silts so that the chert cobbles could be extracted, leaving galleries. This technique mainly took place from the trench. The trench was probably the result of an earlier 'trenching exploitation'[3] of the cobble-bearing layer. On the other hand, the trench may have been the result of cleaning after merging different bell pits. It was during the search for the chert cobble-bearing layer, that the bell pits were dug.

The rubble from the trench was dumped on the northeastern edge of the site probably where the chert cobble layer was naturally exposed. We believe that this natural exposure was the reason why the exploitation started.

Such extractions of mineral substances can be considered as real mining[3]. That this was not continuous mining can be deduced from the fact that prehistoric man was not always sure where to start his digging activities: a shaft was lowered into the rubble from earlier extractions and a search for exploitable chert was carried out in the neighbourhood of the site, such as the site of Nazlet Khater 1, where we excavated a similar bell pit filled with aeolian sand and containing charcoal dated at $31,600^{+3,600}_{-2,500}$ yr BP (GrN-11298). Mining probably only took place when Upper Palaeolithic man came near Nazlet Khater, where he knew fresh chert to be available.

During the 1980 season a narrow (0.2–0.4 m) grave (1.6 m length and 0.6 m deep) was found on the summit of the boulder hill (Fig. 1). Within the grave was a corpse, lying at full length on its back, with the arms stretched along the body. The covering consisted of several boulders, some more than 0.4 m in diameter. The interstices were filled with loose aeolian sand. A tool was laid on the bottom of the grave, at the right of and next to the

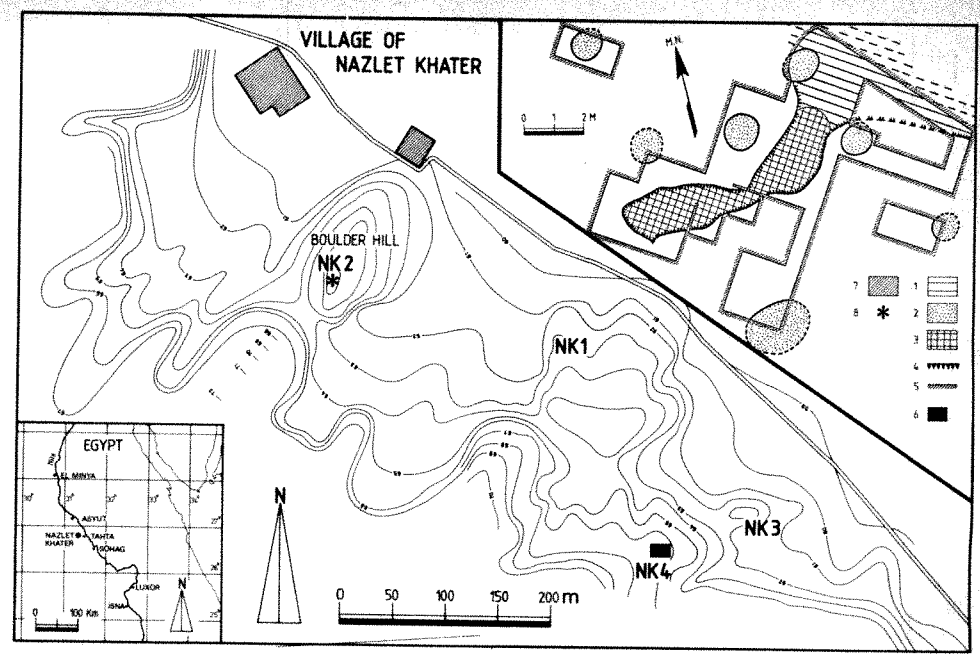


Fig. 1 Site location. 1, Upper Palaeolithic dump area; 2, bell pit; 3, trench; 4, trench wall opening into gallery at the base; 5, excavation limit; 6, chert mining site; 7, enclosed dwelling; 8, grave on the boulder hill.

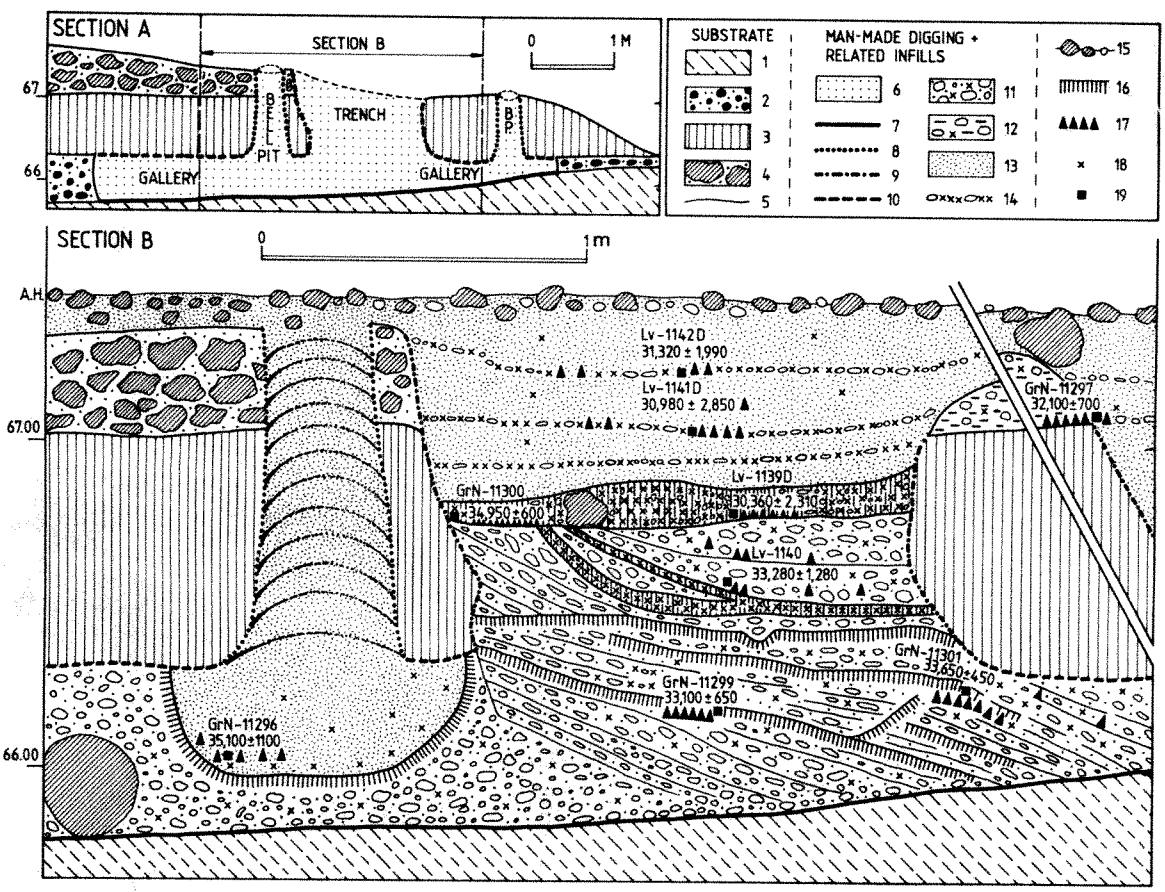


Fig. 2 General (A) and composed (B) section of the site Nazlet Khater 4. 1, Greenish silts and fine sands; 2, Nile gravels; 3, brown silts; 4, wadi deposits; 5, lithological contacts; 6, extent of the exploitation; 7, base of the mining activities; 8, walls of the bell pit; 9, walls of the trench; 10, gallery roof; 11, man-made gravel infills; 12, surface dumps; 13, aeolian sands; 14, derived gravels and artefacts; 15, desert pavement; 16, calcrete; 17, charcoal; 18, Upper Palaeolithic artefacts; 19, ^{14}C-dates in yr BP.

cranium. It is a bifacially shaped axe with concave sides and a convex cutting edge (Fig. 3*c*).

From the state of synostosis of most epiphises and other age indicators, the nearly complete skeletons seems to belong to a sub-adult individual, probably a male, with an estimated height of 1.65 m according to the formulae of Olivier *et al.*[4]. Cranial morphology is certainly that of anatomically modern man. We estimate the cranial capacity to be at least 1,400 cm^3. The facial skeleton displays two 'African' characters: fossa praenasalis and strong alveolar prognatism.

The best-preserved bone is the extremely robust mandible (Fig. 4). Its chin is fully developed. Ramus height (Martin 70)[5] is 72 mm and ramus breadth (Martin 71) is 51 mm. This breadth is very large and is matched only by that of the Mauer mandible. The rameal index is 70.83. According to its published photograph, the probably Aterian mandible of Dar-es-Soltan-2[6] (Morocco) must have a rameal index similar to that of the Nazlet Khater man.

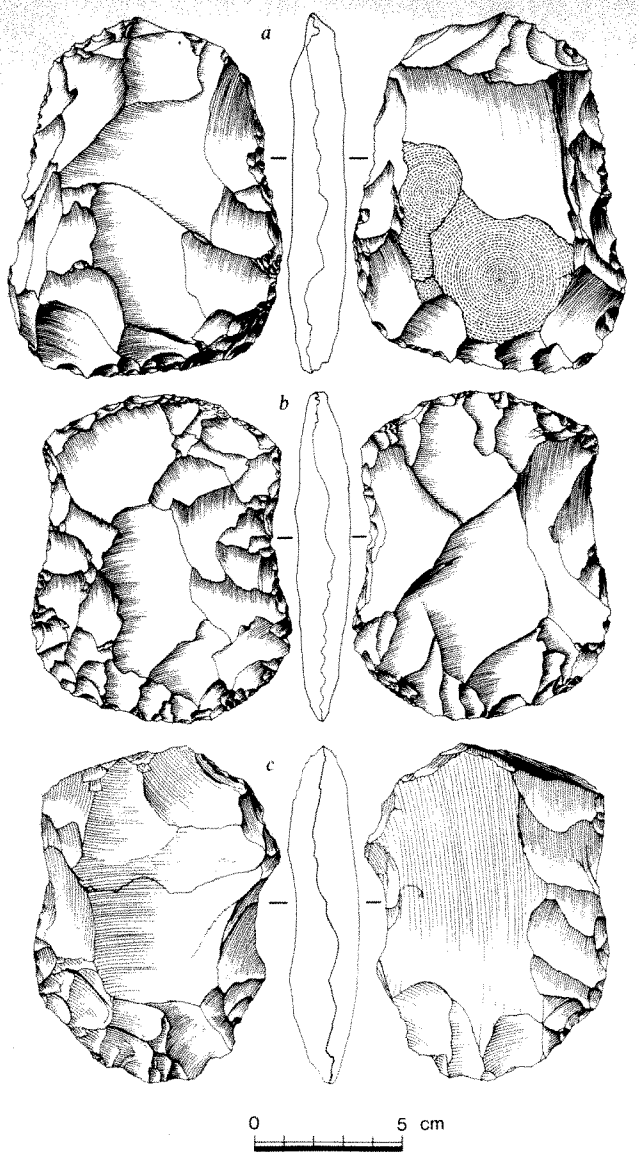


Fig. 3 Upper Palaeolithic axes. *a*, *b*, Axes of NK 4; *c*, burial gift from the grave on Boulder Hill.

The 1982 research shows that the early Upper Palaeolithic blade production on the site of Nazlet Khater 4 is related to chert mining. By about 33,000 yr ago, early Upper Palaeolithic man had acquired typical mining techniques. Indeed, even if the bell pits are only 1.5 m deep, this mining system is comparable with that in general use during the Neolithic[7]. These techniques were so complex that one has to accept that Upper Palaeolithic man chose the bell pit location on the basis of some geological insight.

Extraction pits of raw material to a depth of 0.35 m have been reported from the Nubian Middle Palaeolithic site of Arkin 5[8]. In other parts of the world chert mining was mainly restricted to the Holocene less than 10,000 yr ago. Only ochre extraction[9] took place before that.

Direct dating of the Nazlet Khater man is not possible as no collagen has been preserved in the skeleton bones. However, several arguments indicate that mining site and grave belong to the same prehistoric technocomplex, which is unique for the Nile Valley, and which was dated at 33,000 yr ago.

Indeed, the bifacial axe found near the cranium of the skeleton in the grave is of the same type as those of the mining site. Such axes are not known to occur in the later Palaeolithic of Egypt and predynastic axes are of a different type. Moreover, we have found no traces of predynastic occupation during our intensive regional survey.

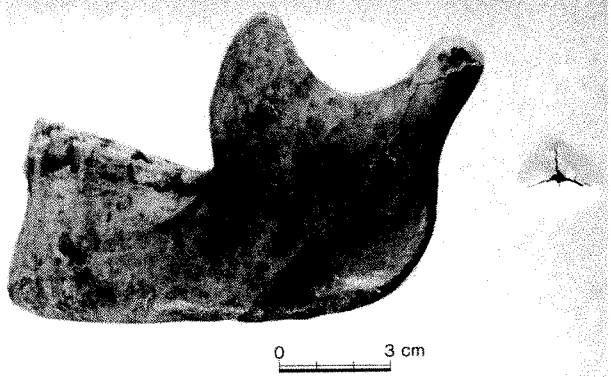


Fig. 4 Mandible (*norma lateralis*) of the Nazlet Khater man.

The boulder hill grave shows no evidence of the burial practices of the later Palaeolithic and predynastic periods, where the interred body is always in a contracted position.

The only prehistoric trenches filled up with aeolian sand are the grave, the mining site and the dated bell pit on the Nazlet Khater 1 site which suggests that a similar date for the aeolian sand in the grave is likely. A final argument for a very old age is the archaic features of the skeleton itself.

All these arguments strongly suggest that the Nazlet Khater man is around 30,000 yr old. In that case the man of Nazlet Khater is the oldest modern man in Africa north of the Equator.

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The chorus-line hypothesis of manoeuvre coordination in avian flocks

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Thousands of birds flying together at high speeds are able to execute abrupt manoeuvres with such precise coordination that some investigators have postulated that 'thought transference'[1] or electromagnetic communication[2] must be taking place. Recently, Davis proposed that coordination is achieved by a 'threshold' number of birds executing 'preliminary movements' which signal to the flock that a turn is imminent[3]. Here I show by analysis of film of dunlin (*Calidris alpina*) flocks that a single bird may initiate a manoeuvre which spreads through the flock in a wave. The propagation of this 'manoeuvre wave' begins relatively slowly but reaches mean speeds three times higher than would be possible if birds were simply reacting to their immediate neighbours. These propagation speeds appear to be achieved in much the same way as they are in a human chorus line: individuals observe the approaching manoeuvre wave and time their own execution to coincide with its arrival.

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The 'chorus-line' hypothesis suggests that manoeuvres are initiated by any bird executing a manoeuvre towards the flock and that subsequent coordination is achieved through visual communication. This leads to the prediction that execution of the manoeuvre by neighbours of the initiator will be delayed by at least their own reaction time but, further away, response times should fall as birds are able to estimate the arrival of the approaching manoeuvre wave. Films taken of human chorus lines indicate that rehearsed manoeuvres, initiated without warning, propagate from person to person approximately twice as fast (107.7 ± 6.8 ms, $n = 3$) as the 194-ms human visual reaction time[4].

Coordinated manoeuvres (defined as those in which interbird response times were faster than laboratory measured reaction times) were investigated by field observations and analyses of 2,000 ft of slow-motion (50 frames s^{-1}) 16-mm film taken of dunlin flocks at Puget Sound, Washington. Manoeuvres were either natural or were induced artificially by shooting an arrow near an airborne flock. Twenty-two coordinated and four non-coordinated manoeuvres, chosen for clarity of detail, were analysed frame-by-frame. Startle reaction times to a light flash were measured in the laboratory using previously reported methodology[5].

In films where initiators and all neighbours were discernible, coordinated manoeuvres were initiated by one or a few individuals (one initiator, $n = 9$; two, $n = 3$; three, $n = 2$; >three, $n = 0$). Birds were considered initiators when they began the manoeuvre before the rest of the flock. In all cases coordinated manoeuvres were initiated by birds banking towards the flock ($n = 22$). When flock members turned away from the flock, the flock either did not follow ($n = 16$), or did so at speeds too low to qualify as a coordinated manoeuvre (84.8 ± 15.5 ms, $n = 4$). Manoeuvres always propagated through the flock in a wave radiating from the initiation site. These waves were observed to travel along every major axis (including back to front), indicating that manoeuvres may be initiated from any region of the flock. They had a mean propagation time from neighbour to neighbour of 14.6 ± 6.7 ms ($n = 9$), considerably lower than the laboratory measured startle reaction time of 38.3 ± 3.1 ms ($n = 110$). Therefore, manoeuvres propagate through flocks in waves travelling at speeds nearly three times faster than possible if flock members are following the actions of adjacent neighbours. However, the first neighbours to respond to the initiator required 67 ± 24 ms ($n = 14$). This mean propagation time from initiator to neighbours was significantly slower than both the mean manoeuvre propagation time (14.6 ms, $P < 0.0001$), and the mean species reaction time (38.3 ms, $P < 0.0001$; Wilcoxon rank sum test).

These results show that manoeuvres initially propagate more slowly than the birds' reaction time and then accelerate to high propagation speeds, as predicted by the chorus line hypothesis. Alternative hypotheses make no explicit predictions about the form of propagation, but imply that manoeuvres can occur in unison. No unison manoeuvres were seen. No preliminary movements which might signal that a turn is imminent[3] were seen, although such movements should be visible on film if they are to be visible to what is often thousands of tightly packed[6] flock members.

Flock members always appear to follow the lead of initiators banking towards the flock. This 'dictatorial' rule by initiators presumably prevents indecision and allows flocks to respond rapidly during attacks by birds of prey, which are a major source of mortality for flocking shorebirds[7]. Birds of prey usually direct their attack toward individuals isolated from the main flock[7,8], and this may well have exerted selection pressure for the evolution of coordinated manoeuvres.

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Auditory receptive fields in primate superior colliculus shift with changes in eye position

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The process by which sensory signals are transformed into commands for the control of movement is poorly understood. A potential site for such a transformation is the superior colliculus (SC), which receives auditory, visual and somatosensory inputs[1–3] and contains neurones that discharge before saccadic eye movements[4–6]. Along the primary sensory pathways, signals coding the spatial location of auditory, visual and somatosensory targets are based on distinctly different coordinate systems, and it is not known whether each type of sensory input uses a separate motor pathway or if they are converted into a common coordinate system in order to share a single pre-motor circuit. Sensory neurones in the SC have spatially restricted receptive fields (RFs) and are organized into maps across the collicular surface[7–9]. Acute experiments have shown a rough correspondence between the spatial positions of RFs of neurones encountered along a single dorsal–ventral penetration of the colliculus, regardless of the modality of the effective stimulus[10–14], suggesting that auditory, visual and somatosensory maps might be in register. However, in these conditions the head-centred auditory system and the retinotopic visual system are aligned because the eyes are in the primary orbital position[15]. Moreover, other data have suggested[16–18] that the primate SC is organized in motor, not sensory, coordinates, although in the cat, eye position was found to have no effect on auditory receptive fields[19]. We therefore sought here to determine what happens to the registration of the auditory and visual maps in the alert, behaving animal. Monkeys, with heads fixed, were trained to make delayed saccadic eye movements to auditory or visual targets from one of three initial fixation points while the activity of single neurones was recorded extracellularly. We found that the auditory receptive fields shifted with changes in eye position, allowing the auditory and visual maps to remain in register.

We trained two monkeys (*Macaca mulatta*) to look to either visual or auditory targets in a completely darkened room. The major objective of the experiment was to plot the RFs of sound-sensitive cells in the SC of alert monkeys while varying the direction of visual fixation. We considered that if the RFs of these auditory neurones were organized in head-centred coordinates, this manipulation would have no effect, whereas if the auditory neurones use oculomotor coordinates, the direction and distance of the sound source from the eyes, not the absolute position of the target in space, would be the crucial factor. Accordingly, the magnitude of the neural responses would depend on both the position of the speaker relative to the head and the position of the eyes in the orbit.

Another objective was to determine whether auditory and visual pathways share a common motor circuit. The activity of some collicular neurones is tightly coupled to saccade onset and is thought to participate in the initiation of spontaneous and

visually-triggered saccades[4–6]. If the neurones that burst before visually-guided saccades also burst before saccades to auditory targets, this would suggest that auditory and visual signals have been converted into the same coordinates by the time they reach the level of the SC.

The monkeys were placed, with their heads fixed, in the centre of a semicircular track[20]. Under computer control, a speaker moved along the track and the hoop rotated so that the target could be placed anywhere on an imaginary sphere surrounding the animal. A light-emitting diode in the centre of the speaker was used to compare auditory and visual responses. Three fixation lights were placed 24° apart along the horizontal meridian.

The auditory stimulus was a broad-band noise burst (20–20 kHz; 90 dB sound pressure level; re: 0.0002 dyn cm^{-2}). The visual stimulus, a green light-emitting diode, subtended a visual angle of 12 arc min. Eye position was monitored using the scleral search coil technique[21]; tungsten microelectrodes were used for extracellular unit recordings.

A delayed saccade task was used to separate temporally sensory and motor activity. At the beginning of each trial, one of the three fixation lights was randomly activated. After a variable time, an auditory or visual target was presented. To receive a liquid reward, the monkey had to maintain fixation of the initial light until it was extinguished and then look to the peripheral target.

For every sound-sensitive cell encountered, the position of the eyes in the orbit had a distinct effect on the neural response to an auditory stimulus. Figure 1*a* illustrates data recorded from a single cell. For these trials, the speaker was located 20° to the right and 6° above the primary eye position. When the gaze was directed towards the left fixation target, the auditory stimulus evoked a vigorous neural discharge with latencies ranging over 40–60 ms. When the monkey was viewing the centre or right fixation lights, identical noise bursts presented in the same spatial location resulted in neural responses that were either markedly attenuated or completely absent.

Figure 1*b* summarizes the data obtained from this cell by plotting the average number of spikes in response to the noise burst against the horizontal position of the speaker (top). The RF of the neurones shifted with the position of the eyes in the orbit. The peripheral boundary of these RFs could not be determined because of the experimental constraint of keeping the targets within the oculomotor range. These data are replotted using the motor error reference system below. The abscissa represents horizontal motor error, or the horizontal component of the eye movements required to look to the auditory targets from the three fixation points. In this case, the data obtained while the monkey viewed the three fixation lights are closely aligned. Thus, the magnitude of the discharge of this neurone was not determined solely by the position of the auditory stimulus in space, but depended jointly on the position of the eyes in the orbit and the target position. Individual cells in the sample varied with respect to the relative strength of the head-centred and motor error signals they carried, but all auditory units analysed ($n = 116$) displayed a shift in RF location with changes in eye position.

Like many visually responsive cells in the intermediate layers of the SC[16], these auditory neurones could not be classified as strictly sensory or motor cells but seemed to exhibit qualities of both systems. Their responses appeared to be sensory in that the discharge was time-locked to the onset of the stimulus. The average latency was ~50 ms for auditory targets and 70 ms for visual ones. Many of the auditory cells could be activated by either auditory or visual stimuli. For these bimodal cells, the vigour of the neural response depended on target modality even though identical motor responses might occur. Some cells discharged more vigorously to auditory stimuli, others to a visual target. In an attempt to dissociate the sensory and motor components of the response, we plotted the auditory fields while the monkey performed a probe task—one that required continuous fixation of the initial light without making a saccade towards the auditory or visual probe stimulus. Most cells rapidly habituated when a motor response was not required. For the remaining cells, the RFs obtained using probe trials were very similar to those generated with the delayed saccade paradigm even though no eye movement occurred. Yet this sound-induced activity cannot be termed 'sensory' because the response depended on the trajectory of the eye movement required to look to the auditory target.

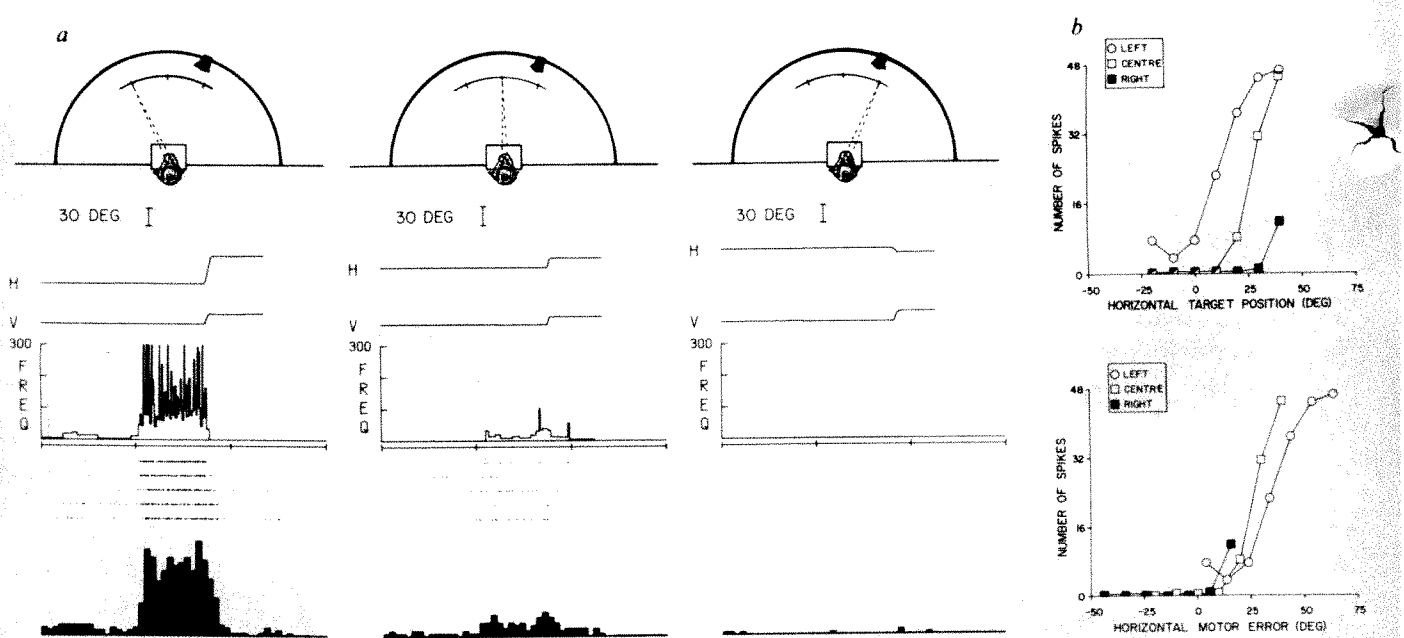


Fig. 1 The effects of eye position on the response of a single SC cell to an auditory stimulus. *a*, The speaker was placed 20° right, 6° up from centre while the fixation position was varied between 24° left (left), centre (centre) and 24° right (right). Time base was 3 s and the target was presented at 1 s. Horizontal (up–right) and vertical eye position traces are shown in the top row. Representations of instantaneous firing rate for single trials are in the second row, followed by rasters showing the unit activity for five trials. The bottom row is a cumulative histogram for these trials. *b*, A summary plot of the auditory RFs for this one cell as a function of horizontal target position (top) showing a shift in the position of the RFs as eye position was varied. The data are replotted below in motor error coordinates—the difference between current eye position and the saccade target. The fields are better aligned in the motor error reference system than in the one based on the position of the target relative to the head.

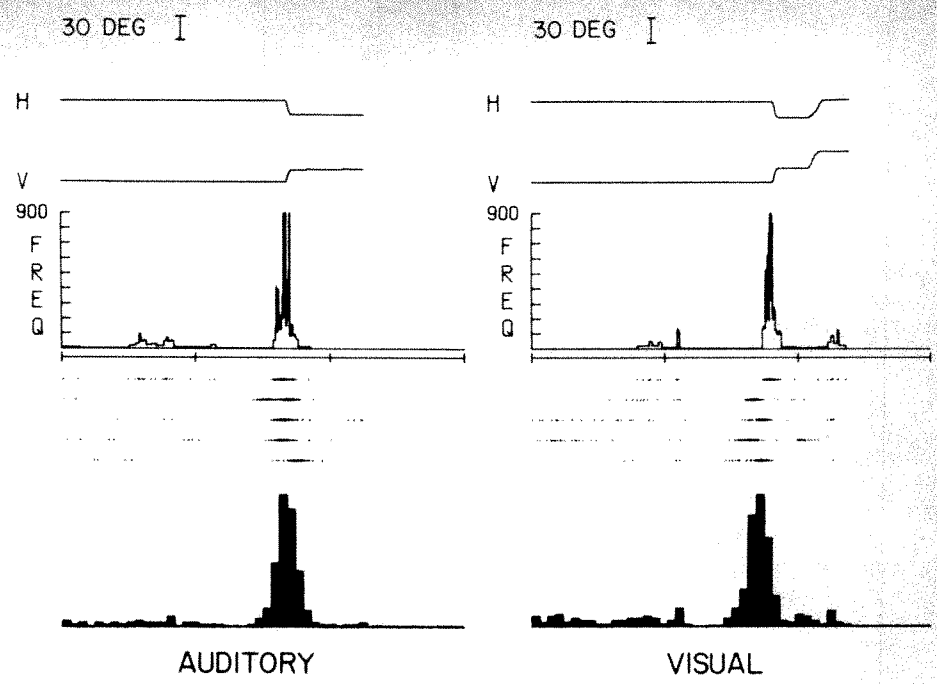


Fig. 2 The convergence of auditory and visual pathways onto a common motor circuit. A single SR burst cell activated before both auditory (left) and visual (right) saccades to a target located 16° left, 10° up. No burst occurred for either modality when a saccade to this same target was initiated from the left fixation point.

As recent work in the cat SC has shown that the position of the pinnae has a significant effect on the spatial properties of auditory cells[22–24], we sought to evaluate the possible contribution of pinna movements in the primate. The RFs were compared in the same cell when the animal was free to move its ears and when the pinnae were mechanically restrained. The field plots were similar in both conditions, indicating that the small pinna movements made by primates are not responsible for the eye position effect observed in this study.

SC neurones that burst before visually initiated saccades were also found to burst before saccades to auditory targets. The activity of a typical saccade-related (SR) burst neurone is illustrated in Fig. 2. With gaze directed at the centre fixation SR burst cells tested in this manner. Thus, at the level of the SC, auditory and visual signals share a common motor circuit.

Our findings suggest that an internal representation of eye position, either efference copy or proprioception, has been subtracted from the head-centred spatial code of auditory targets found elsewhere in the auditory system. As a result, some auditory cells code for the vector of a potential eye movement rather than just the position of a sound source relative to the head. The fact that the auditory cell population we observed displayed a range in the relative potency of motor error and head-centred signals suggests that this transformation may occur in the primate SC. This translation allows the auditory and visual maps in the intermediate and deep layers of the primate SC to share a reference system (motor error) and to converge onto a common motor pathway for the generation of saccadic eye movements.

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Association with persistent neuroleptic-induced dyskinesia of regional changes in brain GABA synthesis

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The movement disorder tardive dyskinesia is a serious side effect of the long-term treatment of schizophrenia with neuroleptic drugs. Similar symptoms to those of tardive dyskinesia have been observed in *Cebus apella* monkeys following long-term treatment with neuroleptic drugs[1,2], and these monkeys may therefore be a useful animal model of tardive dyskinesia. Motor defects have persisted in these dyskinetic monkeys for periods of 1–6 yr after the cessation of neuroleptic treatment. We report here that in three regions of the brains of dyskinetic monkeys (substantia nigra, medial globus pallidus and subthalamic nucleus) glutamate decarboxylase activities and γ-aminobutyric acid (GABA) levels are reduced relative to control monkeys that had been treated with neuroleptics but which showed none of the symptoms of tardive dyskinesia. These results suggest that alterations in the GABA neurone system are involved in neuroleptic drug-induced tardive dyskinesia.

Nineteen *C. apella* monkeys (10 male, 9 female) weighing 1.6–4.2 kg (mean 2.9 kg) were used in the present experiments. Twelve monkeys received chronic neuroleptic treatment: haloperidol (nine animals) or fluphenazine (three) for 3–6 yr, while seven were untreated controls. Intramuscular (i.m.) injections of haloperidol decanoate (1–10 mg per kg) or fluphenazine decanoate (4–10 mg per kg) were given at 3-week intervals. After varying treatment periods, six of these monkeys (three injected with haloperidol, three with fluphenazine) developed persistent choreic dyskinetic movements in the trunk, neck and extremities (one monkey also had oral dyskinesia). The frequency of dyskinetic movements and symptom intensity were rated during 2-min sessions[3]. When these measurements were made at 1 and 60 days after the final neuroleptic injection, only slight changes in intensity of dyskinetic symptoms were evident.

Throughout the present study three groups of animals were compared: untreated controls ($n = 7$), neuroleptic-treated controls ($n = 6$) and dyskinetic animals ($n = 6$). Before the neuroleptic-treated animals were killed the regular depot drug was substituted by a single i.m. injection of non-depot haloperidol or fluphenazine (0.05 mg per kg). Two months after the final injection, the animals were killed under brief methohexital anaesthesia. Within 10–12 min of killing the brains were removed and stored at −80 °C. Microdisks (1.075 mm^3) were later punched from successive coronal sections prepared from the frozen brains. Glutamic acid decarboxylase (GAD) activity was measured as $^{14}CO_2$ formation from [1-^{14}C]glutamic acid during 30 min in the presence of pyridoxal-5-phosphate[4]. GABA, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were assayed by gas chromatography–mass spectrometry in the selected ion-monitoring mode using deuterium-labelled internal standards[5,6]. In most animals only the left half of the brain was analysed, but in six (two untreated controls, one neuroleptic-treated control and three dyskinetic monkeys) both sides were compared (one of the dyskinetic monkeys had one-sided symptoms).

Mean levels of transmitter markers at each brain site were compared between the three groups of monkeys using independent Student's *t*-tests. In addition, all values obtained for a certain brain nucleus were combined and means for each nucleus were compared using *t*-tests between the three groups. In one case (see Fig. 1) sign-tests and χ^2-tests were used.

The neuroleptic-treated monkeys which did not develop dyskinesia (neuroleptic-treated controls) did not differ significantly from untreated controls in any brain region with regard to GAD activity and levels of GABA, HVA or DOPAC, whereas the dyskinetic animals showed a characteristic pattern of regional changes. Table 1 shows regional GAD activity and GABA levels for untreated controls and per cent changes within both neuroleptic-treated groups. The dyskinetic monkeys showed a reduced GAD activity in the substantia nigra, nucleus subthalamicus and medial globus pallidus and GABA was low in the substantia nigra and n. subthalamicus. In all other brain areas group differences for GAD and GABA were insignificant. In Fig. 1 the mean nigral GAD activity for each individual monkey has been plotted against the number of animals. The dyskinetic group differed from the controls (χ^2 tests, $P < 0.001$), verifying that the number of animals was sufficiently large to allow generalized conclusions regarding relations between state (dyskinetic versus non-dyskinetic) and biochemical changes.

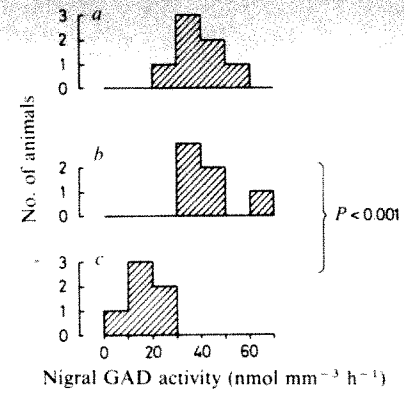


Fig. 1 Distribution of individual (mean) GAD activity levels in substantia nigra in three groups of monkeys: *a*, untreated controls; *b*, neuroleptic-treated controls; and *c*, dyskinetic animals.

GAD activity in substantia nigra was generally equally high on both sides; a conspicuous exception was found in one animal with unilateral dyskinesia in the left hand and arm. In this animal there was a depression of GAD activity only in the opposite (right) nigral area (15.3 nmol mm^{-3} h^{-1}), whereas the ipsilateral nigra had a mean value in the normal range (41.0 nmol mm^{-3} h^{-1}).

The HVA levels were significantly reduced in the caudate nucleus and substantia nigra of dyskinetic animals (Fig. 2). In

Table 1 Regional brain GAD activity and GABA concentrations (±s.d.) in seven untreated controls, and per cent deviations in six neuroleptic-treated controls and six monkeys with neuroleptic-induced persistent dyskinesia

Area	GAD			GABA		
	nmol mm^{-3} h^{-1} UC	%Δ NC	%Δ NPD	ng mm^{-3} UC	%Δ NC	%Δ NPD
Cortex	9.9 ± 1.6	−7	−5	255 ± 35	−10	−11
Caudate	11.7 ± 2.1	+4	0	328 ± 93	−3	−6
Putamen	12.2 ± 1.5	−1	0	393 ± 56	−6	−6
Accumbens	19.2 ± 2.6	+9	+5	791 ± 25	0	−6
Globus pallidus lateralis	36.5 ± 7.0	+2	−16	1,210 ± 42	−5	−9
Globus pallidus medialis	36.9 ± 2.4	+4*	−26‡	1,420 ± 26	−5	−5
Amygdala	8.6 ± 0.9	−13	−12	287 ± 53	−10	−8
Hypothalamus	21.5 ± 4.4	−3	−8	735 ± 97	−9	+2
Thalamus	9.8 ± 1.7	−4	−4	322 ± 59	+2	+3
Nucleus subthalamicus	9.7 ± 2.3	+5*	−32§	504 ± 79	−20	−47‡
Substantia nigra	45.8 ± 6.6	+18†	−57‖	1,000 ± 133	−9	−20‡
Nucleus ruber	8.9 ± 1.9	+17	+19	374 ± 37	−3	+2
Geniculate lateralis	8.4 ± 1.0	+4	−11	196 ± 21	+10	+4
Superior colliculus	13.4 ± 2.9	−18	+10	545 ± 39	−3	−4
Formatio reticularis	18.0 ± 3.9	−7	−5	612 ± 29	−10	−9
Pons	10.5 ± 5.1	−10	−17	379 ± 147	0	−8

UC, Untreated controls; NC, neuroleptic-treated controls; NPD, monkeys with neuroleptic-induced persistent dyskinesia. The NC group did not significantly differ from UC at any brain site.

* $P < 0.01$; † $P < 0.001$, NPD compared with NC.

‡ $P < 0.05$; § $P < 0.01$; ‖ $P < 0.001$, NPD compared with UC.

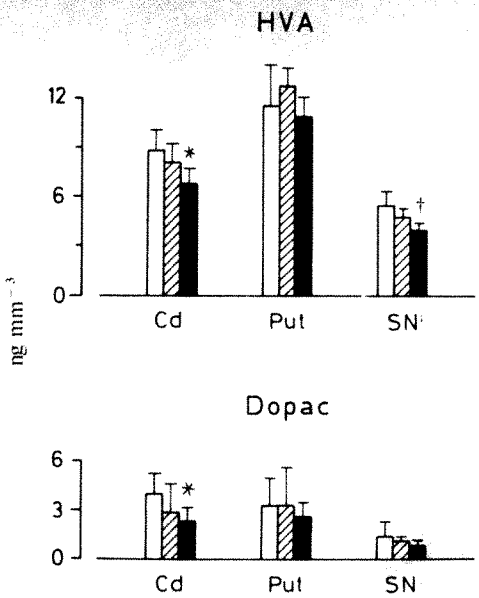


Fig. 2 HVA and DOPAC levels in the caudate nucleus (Cd), putamen (Put) and substantia nigra (SN) of seven untreated controls (open columns), six neuroleptic-treated controls (hatched columns) and six dyskinetic monkeys (solid columns). Values plotted are means ± s.d. Significance levels: * $P < 0.05$, † $P < 0.01$.

the caudate there was also a reduction of DOPAC. In all striatal areas studied (20 in the caudate, 16 in putamen) the mean levels of transmitters in dyskinetic animals were lower than in neuroleptic-treated controls. The greatest depression was found in corpus nuclei caudati and in some nigral areas (unfortunately the technique did not allow us to differentiate between nigra reticulata and compacta).

When striatal dopamine transmission is blocked by neuroleptic drugs this, by trans-synaptic mechanisms, elicits a series of changes in activity elsewhere in the brain[7]. The turnover of GABA in the substantia nigra is lowered[8], which in turn has effects on nigral efferents under tonic inhibitory control from the striatonigral GABA neurone system[9–12]. In the present experiments we noted a lowered GAD activity and/or reduced GABA levels in three brain areas: the substantia nigra, globus pallidus medialis and the subthalamic nucleus. These signs of a reduced GABA function in three brain nuclei were evident only in dyskinetic animals and are probably due to longstanding local depression of GABA neurone activity. Thus, even if all monkeys display local GABA depression during neuroleptic drug administration, only the dyskinetic animals fail to recover their GABA function after withdrawal of the drug.

Further evidence of a connection between dysfunction of nigral GABA and movement disorders is provided by the observation that intra-nigral injection of GABA antagonists precipitates oral dyskinesia in rats[13]. In this context it is noteworthy that low nigral and striatal levels of GABA and GAD have been reported in patients dying of Huntington's chorea, another chronic dyskinetic condition[14,15]. Low nigral GAD activities have also been reported in subjects suffering from Parkinson's disease—again this is secondary to the impaired striatal dopamine transmission, since L-dopa seemed to retain nigral GAD activity at normal levels[16]. Analogously, intrastriatal injection of dopamine has been reported to increase glucose utilization in those areas (substantia nigra, globus pallidus medialis and subthalamic nucleus) where we noticed a reduction of GAD activity in our dyskinetic monkeys[17]. A reduction of nigral GAD activity has also been found in rats made dyskinetic by chronic haloperidol administration[4]. GAD is known to be preferentially located in GABA neurone terminals[18]. Whether the low GAD activity in the three brain areas reported here is due to a reduced tissue density of GABA terminals (that is, caused by neuronal cell degeneration) or a reversible functional depression, must await histological evidence.

The reduced nigral and striatal dopamine metabolism in dyskinetic monkeys would seem incompatible with a dysfunctioning striatonigral GABA system if this is regarded as a link in a negative feedback control of nigrostriatal dopamine nerves. If this feedback loop hypothesis[19–23] were correct, an impairment of its GABAergic link would be expected to increase rather than reduce the turnover rate of dopamine. However, this hypothesis has been disputed and the possibility of a positive feedback has occasionally been proposed[24]. The finding that GABA agonists injected into the substantia nigra reticulata induce contraversive turning and an increased turnover of dopamine in the striatum[12,25–28] supports the notion that dopamine neurones are subjected to positive feedback.

Supersensitivity in striatal dopamine receptors has been suggested as the main cause of tardive dyskinesia[29,30]. However, when rats are given neuroleptic drugs they develop such supersensitivity to dopamine agonists even after a single injection[31]. Further, this phenomenon is not irreversible and thus poorly if at all related to dyskinetic movements continuing for months after neuroleptic drug withdrawal. The present paper instead seems to link persistent dyskinesia with a weakness in the GABAergic neurone system induced by trans-synaptic mechanisms in neurones remote from the striatal dopamine receptor.

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Chronic neuroleptic treatment enhances neurotensin receptor binding in human and rat substantia nigra

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Antischizophrenic neuroleptic drugs interact with brain dopamine systems in producing both therapeutic and unwanted side effects[1]. Short-term administration of neuroleptics may produce a parkinsonian, hypokinetic syndrome mimicking symptoms of dopamine depletion, and presumably caused by dopamine receptor blockade[2]. Paradoxically, some 20% of patients treated chronically with these agents develop tardive dyskinesia. The adventitious movements typical of this condition mimic symptoms of dopamine excess despite continuing receptor blockade[3–5]. Dopamine receptor supersensitivity does develop in such conditions, but its extent does not correlate well with the presence or absence of tardive dyskinesia[6]. Exploration of neuroleptic-induced alterations in other dopamine-associated systems may thus provide insight into these processes. Dopamine-containing cells of the substantia nigra, prominently implicated in the motor side effects of neuroleptics, possess dense concentrations of receptors for the putative peptide neurotransmitter, neurotensin[7–9]. Here we report that these receptors are substantially increased in both rats and humans after chronic treatment with neuroleptic drugs, and discuss possible implications of this finding for our understanding of neuroleptic actions.

Neurotensin is a central nervous system neurotransmitter candidate anatomically disposed to interact with dopaminergic neurones[10–13]. In particular, several lines of evidence indicate that there are dense localizations of neurotensin receptors on neurones in the substantia nigra from rats and human tissue. Autoradiographic studies demonstrate dense concentrations of neurotensin-binding sites in the substantia nigra that are depleted by rat and human lesions destroying dopamine-containing cells[7–9,14]. The neurotensin receptors appear to be physiologically active. Intracisternal neurotensin administration depletes striatal dopamine levels[15], while microiontophoretic application of the peptide selectively excites nigral compacta neurones[16]. Neurotensin potently enhances dopamine release from striatal slices[17]. We have examined these nigral neurotensin receptors in rats and humans with and without chronic neuroleptic (haloperidol) treatment.

Sprague–Dawley rats (200 g) were injected subcutaneously (s.c.) daily for 4 weeks with 0.5 mg per kg of either haloperidol or vehicle alone, according to the regimen of Burt *et al.*[18]. Frozen midbrain slabs taken from brains of drug-treated patients with schizophrenia and from neurologically normal controls (given by Drs Wallace Tourtellotte and Peter Whitehouse) were maintained at −70 °C until sectioning (see Fig. 3 legend for details). Neurotensin receptor autoradiography was performed as described elsewhere[7]. Briefly, a 60-min incubation with 4 nM ^{3}H-neurotensin (86 Ci mmol^{-1}; NEN) was followed by two 5-min washes, then rapid drying of the tissue. Serial sections incubated in medium containing 5×10^{-6} M unlabelled neurotensin (Peninsula) and ^{3}H-neurotensin served as 'blank' controls. Autoradiography was performed using the emulsion-coated coverslip technique (Kodak NTB 3). After 6 weeks of exposure, the emulsion was developed and the tissue stained with toluidine blue. For 'saturation' analysis, sections from two haloperidol-treated rats, two control rats, two neuroleptic-treated schizophrenics, and two neurologically normal control patients were incubated in 10^{-7} M labelled neurotensin, as

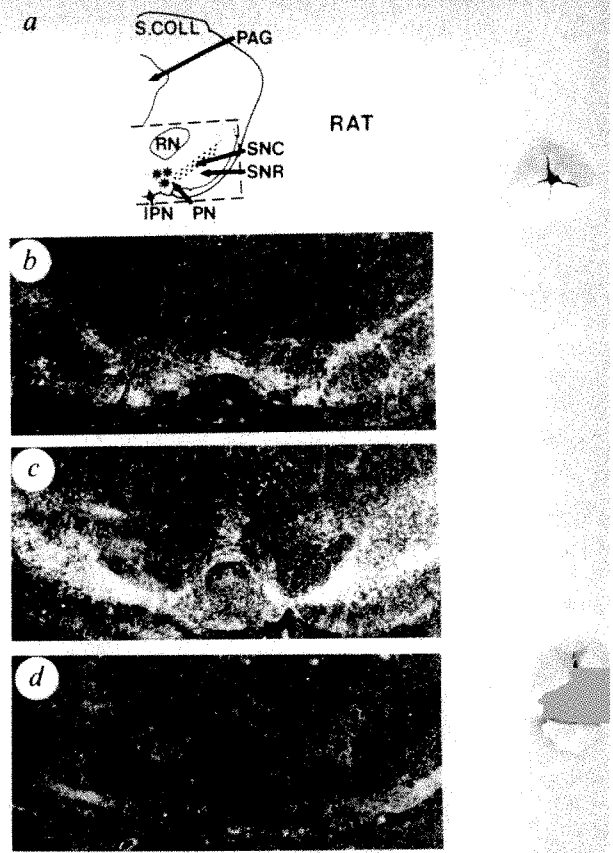


Fig. 1 Neurotensin receptors in rat basal midbrain. *a*, Diagram of the midbrain area shown in *b–d* (the dashed box represents the left half of these photographs). SNR, substantia nigra pars reticulata; SNC, substantia nigra pars compacta; IPN, interpeduncular nucleus; RN, red nucleus; PAG, periaqueductal grey; PN, nucleus paranigralis; S. COLL, superior colliculus. *b*, Darkfield photomicrograph of ^{3}H-neurotensin autoradiogram of the basal midbrain from a control saline-injected rat. *c*, Same as *b* for haloperidol-injected rat. *d*, 'Blank' section adjacent to *a*, incubated with 5×10^{-6} M unlabelled neurotensin and 4 nM ^{3}H-neurotensin to establish a control. ×32.

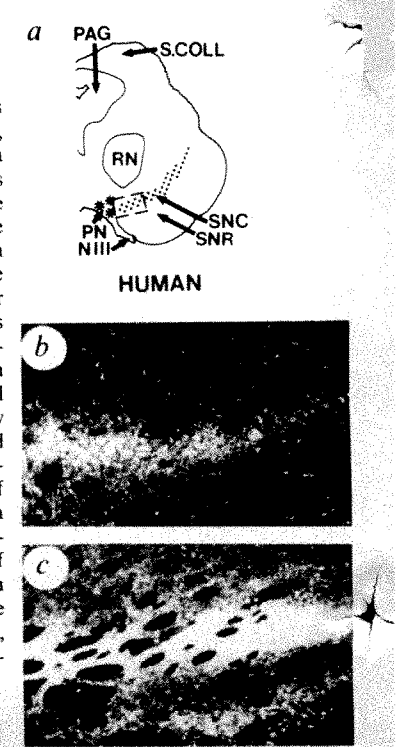


Fig. 2 Neurotensin receptors in human basal midbrain. *a*, Diagram of the midbrain area shown in *b*, *c*. (Box represents the approximate location of the photographs; it is smaller here than in Fig. 1 as the human nigra is ~5 times larger than the rat nigra.) NIII, oculomotor nerve. Other abbreviations as for Fig. 1. *b*, Darkfield photomicrograph of ^{3}H-neurotensin autoradiogram of the basal midbrain from a neurologically normal human. *c*, Darkfield photomicrograph of ^{3}H-neurotensin autoradiogram of the basal midbrain from a schizophrenic, neuroleptic-treated human. Broadening of the zone of dense neurotensin receptor grains is evident in the human nigra, as in the rat nigra, following neuroleptic treatment. ×36.

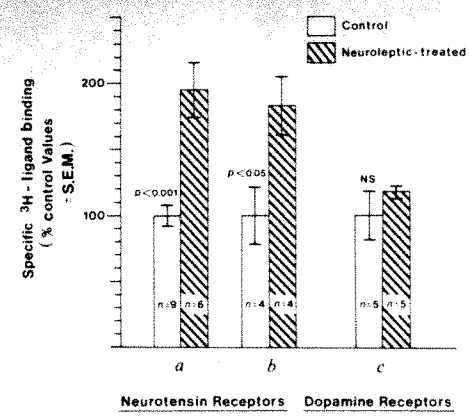


Fig. 3 Histogram of neurotensin (*a* and *b*) and dopamine (*c*) receptor densities in substantia nigra of normal and neuroleptic-treated humans (*a*) and rats (*b* and *c*). *a*, Specific ^{3}H-neurotensin receptor binding levels in substantia nigra specimens from neuroleptic-treated schizophrenic patients and neurologically normal humans without known neuroleptic treatment. Mean age of the control patients was 51; that of the schizophrenics 50. Mean postmortem interval for control brains, 22 h; for schizophrenics, 29 h. The age, postmortem interval, disease duration, cause of death and medication regimen of the six drug-treated human schizophrenic patients were as follows: S1: 36 yr, 27 h; >15 yr, ? overdose, fluphenazine/thioridazine/doxepin/molindon. S2: 36 yr, 41 h, ? 15 yr, gunshot wound, chlorpromazine/thiothixene/fluphenazine/trifluoperazine. S3: 46 yr, 24 h, 20 yr, myocardial infarction, fluphenazine. S4: 51 yr, 29 h, "years", aspiration, loxapine. S5: 64 yr, 39 h, 12 yr, bowel perforation, chlorpromazine/trifluoperazine. S6: 67 yr, ~12 h, "years" ?overdose, ?haloperidol. The age, postmortem interval and cause of death for nine neurologically normal control patients without histories of neuroleptic use were as follows. N1: 30 yr, 33 h, multiple trauma. N2: 36 yr, 19.5 h, myocardial infarction. N3: 40 yr, 42 h, myocardial infarction. N4: 40 yr, 19 h, sudden death. N5: 42 yr, ~18 h, myocardial infarction. N6: 61 yr, 23 h, myocardial infarction. N7: 65 yr, ~12 h, myocardial infarction. N8: 66 yr, 16 h, gunshot wound. N9: 77 yr, ~12 h, ruptured aorta. *b*, Specific ^{3}H-neurotensin receptor binding levels in substantia nigra specimens from rats treated for 1 month with 0.5 mg per kg per day of either haloperidol s.c. or vehicle alone. *c*, Dopamine receptor binding (ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronapthalene)–displaceable ^{3}H-spiroperidol binding) to rat substantia nigra sections adjacent to those assayed for ^{3}H-neurotensin binding in *b*. (Data from G.R.U., P. Whitehouse, D. Price and M.J.K., in preparation.)

described above, and exposed to coverslips for 3 weeks. Dopamine receptors were labelled as described elsewhere[19]. We counted grains overlying the substantia nigra zona compacta, ~400 μm from its medial border. Three 1,000-μm^2 regions of each substantia nigra were counted manually in each case. The data presented here derive from the substantia nigras of nine normal humans, six drug-treated schizophrenic patients, five saline-injected rats and five haloperidol-injected rats. Values for adjacent 'blank' sections from these same regions were subtracted from the values obtained to yield 'specific' grain counts. Statistical calculations (Student's *t*-test, Pearson correlation coefficient) were performed using a Hewlett–Packard programmable calculator.

In the control human brains, and in rat brains, receptor distributions in the basal midbrain were similar to those reported previously[7–9]. High densities of receptor grains overlying the substantia nigra and ventral tegmental area contrasted with much lower levels over the cerebral peduncles and red nucleus (Figs 1*b*, 2*b*). These high grain densities were 6–10 times the levels noted in adjacent 'blank' sections incubated in the presence of unlabelled neurotensin (Fig. 1*d*). Values obtained for human blank samples were also less than one-sixth of total nigral binding. Densities of receptors in human control cases did not correlate significantly with either patient age or postmortem interval ($P > 0.1$ in each case).

Binding of labelled neurotensin in the substantia nigra was substantially increased in both rats and humans treated with neuroleptics (Figs 1*c*, 2*b*). There was almost a doubling of receptor densities in both species, with only slight overlap between normal and drug-treated values ($P < 0.05$ for rats; $P < 0.01$ for humans) (Fig. 3*a*, *b*).

Saturation analysis suggested that this alteration in receptor binding was principally due to alterations in receptor number, as we found the same relationship between treated and untreated individuals. Human schizophrenic tissue showed 1.8 times the binding in normal control tissue, while drug-treated rats revealed 1.9 times, however, control binding. Our ability to detect subtle changes in the affinity constant, K_D, using this paradigm is, however, limited.

Could these results be due to direct neuroleptic influences on peptide binding? In *in vitro* binding assays performed using homogenates or tissue sections, concentrations of haloperidol up to 10^{-5} M were ineffective in displacing or augmenting neurotensin binding, making this an unlikely possibility (G.R.U., unpublished data)[10].

Recently, Mazella *et al.* described[20] high- ($K_D = 0.1$ nM) and low- ($K_D \sim 4$ nM) affinity binding sites for ^{125}I-neurotensin; we might expect ~50% occupancy of lower affinity and ~97% occupancy of higher affinity sites in our binding conditions. It is possible that we have labelled both of these sites in our studies[20].

These results may have implications for understanding receptor regulation, and might provide insight into neuroleptic drug effects. Chronic administration of neuroleptic, dopamine receptor-blocking drugs has been associated with several brain changes. Nigrostriatal receptors for dopamine, especially the D-2 subtype, and for cholecystokinin peptides are increased by ~20% by neuroleptics[6,18,21]. We have observed similar quantitative changes in autoradiographic dopamine receptor binding in our rat brains, although these alterations did not reach statistical significance with our small sample (G.R.U., P. Whitehouse and M.J.K., in preparation; Fig. 3*c*). In the present study, neuroleptic-induced neurotensin receptor changes were of substantially greater magnitude than alterations in the density of other receptors influenced by these drugs. Recent electron microscopic studies revealing increased numbers of terminals on nigral dendrites in neuroleptic-treated animals could provide a possible anatomical correlation with the present results[22]. Interestingly, the increased population of nerve endings possesses properties characteristic of (though not diagnostic of) peptide-containing terminals[23–26]. Conceivably, presynaptic as well as postsynaptic peptidergic mechanisms could be involved. Neurotensin levels are elevated in some brain areas in neuroleptic-treated rats and schizophrenics, though nigral peptide levels have not been examined[27,28].

These results seem to provide an example of 'autoregulatory' responses extending beyond changes in drug-blocked receptors to include other receptor types on the same cell. The increased density of neurotensin receptors could lead to increased excitability of dopamine cells, and partially supercede the effects of dopamine receptor blockade. Whether such an effect could occur at the level of the individual cell, or whether it requires polysynaptic feedback circuits such as those provided by striatonigral projections awaits further study.

The magnitude of the neuroleptic-induced neurotensin receptor change and its occurrence in both rat and human brains are consistent with a possible role for these peptide receptor alterations in the clinical expression of long-term neuroleptic effects such as tardive dyskinesia. Augmented neurotensin receptor densities on nigrostriatal neurones could possibly lead to increased dopamine release from these neurones, and hence to motor symptoms of dopamine excess. Conceivably, elucidation of neuroleptic-induced alterations in nigral neurotensin receptors could enhance our understanding of the motor side effects of frequently used neuroleptic drugs.

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Cation selectivity of light-sensitive conductance in retinal rods

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Vertebrate photoreceptors respond to light by a membrane hyperpolarization which is thought to result from the decrease of a Na-selective conductance in the outer segment[1–6]. One hypothesis is that light increases intracellular free Ca which reversibly blocks the Na conductance[7,8]; at least part of this Ca is then extruded to the cell exterior by a Na–Ca exchanger at the plasma membrane[9–14]. We describe experiments here which show that the light-sensitive conductance in rods is also highly permeable to K. While external Na acts to keep the conductance open, external K tends to keep it closed, both actions probably involving the Na–Ca exchanger. The conductance is also permeable to the monovalent cations Li, Rb and Cs and the divalent cations Ca, Sr and Ba. The ability of both Na and K to go through the light-sensitive conductance explains the long-standing puzzle as to why the reversal potential for the light response is at 0 to +10 mV[15–17].

Under IR light a piece of retina from a dark-adapted toad (*Bufo marinus*) was finely chopped in Ringer's solution, yielding some isolated rods with intact outer and inner segments. The light-sensitive current from one of these rods was recorded by sucking its inner segment into a close-fitting glass pipette containing Ringer's solution while its outer segment was exposed to perfusing solution[13,14]. The solution exchange time as judged from junction currents[14] was about 200 ms, rapid enough to study the light-sensitive conductance before it collapsed upon removal of all external Na[14].

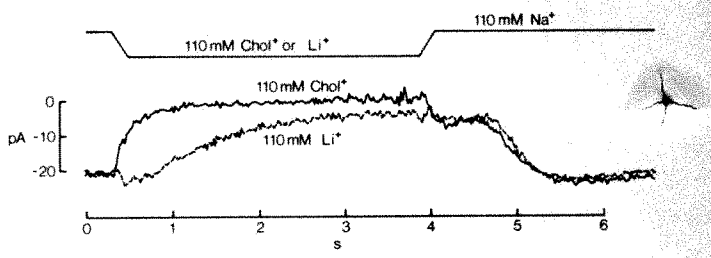


Fig. 1 Effects on the light-sensitive current in toad rod of complete replacement of Na in Ringer's by choline and Li. External Ca was maintained at 1 mM throughout. Recordings were made from the same rod with a current-to-voltage converter connected to the suction pipette. Time courses of solution changes, as indicated by junction currents, are shown above the traces.
Methods: The valve which controlled solution changes was operated pneumatically and with electronic timing so that the solution changes could be reproduced exactly many times. Traces were obtained from total recorded current (light-sensitive current plus junction current), measured in darkness, minus junction current, measured in a repeated run in bright light. Outward light-sensitive current across the outer segment is plotted upwards. In addition to 110 mM Na, choline or Li, the solutions also contained 2.5 mM K, 1.6 mM Mg, 1.0 mM Ca, 117.7 mM Cl, 5 mM tetramethylammonium (TMA)-HEPES, 5 mM dextrose and were buffered to *p*H 7.65. The choline trace is averaged from two dark minus two light trials; the Li trace is from the difference between single dark and light trials. All recordings were low-pass filtered at 100 Hz, 8-pole, and bright steady light for junction current measurements delivered 80–189 photons (500 nm) $\mu m^{-2} s^{-1}$, unpolarized.

The solid trace in Fig. 1 shows an experiment in which all Na in the perfusing Ringer's was briefly replaced by choline, a cation with little or no ability to go through the light-sensitive conductance[13,14,18]. The initial rapid reduction in current upon Na removal indicates removal of most or all of the Na current, while the small initial current jump upon restoring Na probably represents Na current going through a substantially lower light-sensitive conductance caused by the failure of choline to drive the Na–Ca exchanger and maintain open conductance. The delayed secondary increase in current probably reflects the gradual reopening of the conductance due to the reactivation of the Na–Ca exchanger by Na, which leads to lower internal Ca. The dashed trace in Fig. 1 was obtained from the same cell with Li replacing Na. There was no initial drop but rather a slight increase in current upon Li substitution, but thereafter the current did decline to a low level. Upon restoring Na there was no initial jump in current, but the secondary rise was identical to the choline case. These results suggest that Li goes through the light-sensitive conductance as well as or slightly better than Na but that it cannot run the Na–Ca exchanger. In the squid axon Li also cannot substitute for Na in driving the exchanger[19].

Figure 2*a* shows a similar experiment in which K completely replaced Na. Like the Li case, there was an excess of inward light-sensitive current compared with choline replacement that could only be attributed to K. The small initial decline in current indicates that the conductance is somewhat less permeable to K than to Na, while the secondary decline again suggests a progressive reduction in the conductance. From the initial current reductions in choline and in K the permeability ratio K:Na was estimated to be about 0·6. Besides carrying current, external K also tended to reduce the light-sensitive conductance. Figure 2*b* shows that when 20 mM or 55 mM Na was replaced, the steady-state current was actually smaller in K than in choline, even though K could carry substantial current. This blocking action of K seems to arise from inhibition of the Na–Ca

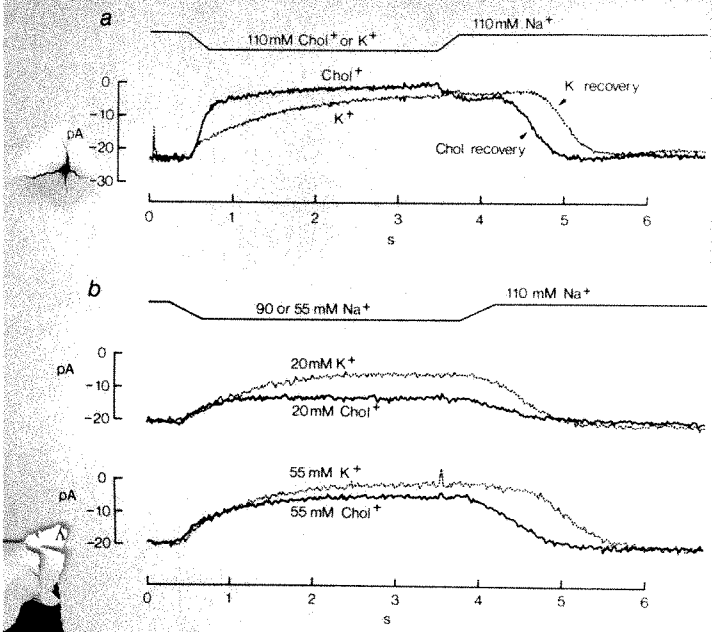


Fig. 2 Effect on rod light-sensitive current of the replacement of Na in Ringer's by K. 1 mM external Ca throughout. *a*, Complete replacement. *b*, Partial replacements. Two separate cells in *a* and *b*. The junction currents are already subtracted in each trace. All other ionic concentrations are the same as in Fig. 1 (including the extra 2.5 mM K). In *a*, the choline record is averaged from the differences between two dark and two light trials (alternated) while the K record is averaged from the differences between four dark and four light trials. In *b*, each of the choline and K records is averaged from the difference between two dark and two light trials. Small spikes in lower records of *b* are from incomplete subtraction of valve movement artefacts. Temperature 24.5 °C in both cases.

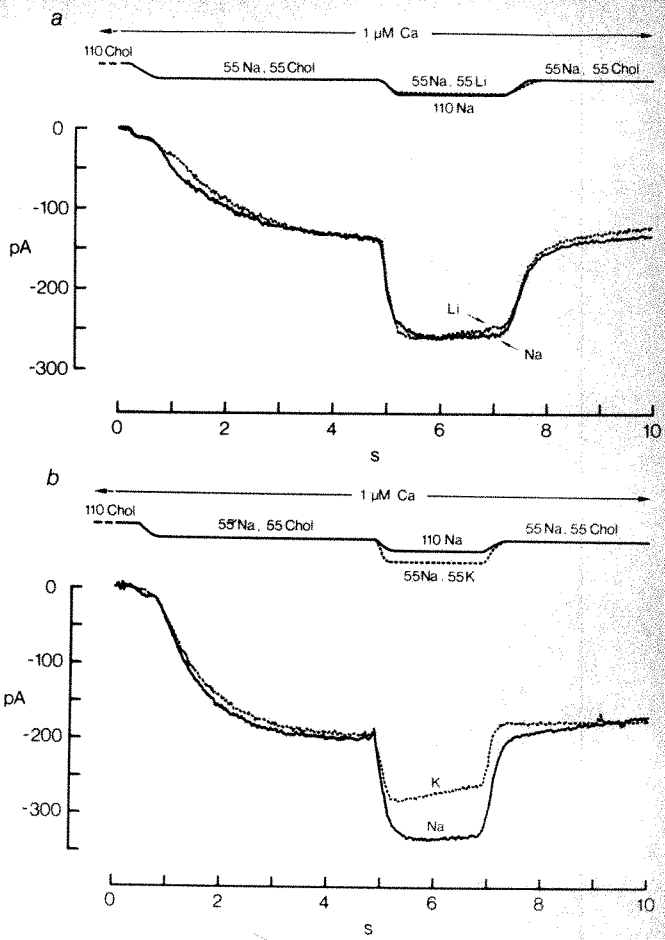


Fig. 3 Comparison of light-sensitive currents carried by Na, Li and K after the conductance was increased or 'primed' by a solution containing 55 mM Na, 55 mM choline and 1 μM Ca. Two separate cells in *a* and *b*. Junction currents, shown magnified above the light-sensitive currents, indicate the time courses of solution changes. The junction currents are already subtracted in each trace. All traces are from single trials. In addition to Na, choline, Li and K in various isotonic substitutions, the buffered 1 μM Ca solutions contained 2.5 mM K, 1.5 mM Mg, 0.093 mM Ca, 2 mM Mg-EDTA, 5 mM TMA-HEPES, 5 mM dextrose and were buffered to *p*H 7.6 (see ref. 14 for Ca buffering).

exchanger, as suggested by the increased delay in recovery after high K exposure (Fig. 2*b*). Indeed, the recovery after 55 mM Na, 55 mM K was as much delayed as that after 0 Na, 110 mM choline (see Fig. 2*a*), when the Na-dependent Ca efflux is presumably fully inhibited. Part of this K inhibition is probably due to a membrane depolarization effect on the exchanger, resulting from high K leaking into the pipette and affecting the inner segment, but there may also be a direct inhibition by K on the exchanger.

It has previously been shown that low external Ca substantially increases the light-sensitive current in rods[7,13,14], apparently by reducing internal free Ca[14]. This provides a more striking way to demonstrate passage of Li and K through the conductance. In Fig. 3*a* and *b* the outer segment was initially exposed to a 'priming' solution containing 55 mM Na, 55 mM choline and 1 μM Ca[14], which caused the light-sensitive current to climb slowly to a high level. At the plateau level an additional 55 mM Na, Li or K was added and the resulting current increases were compared. From the slopes or initial peaks of the current increases, the Li:Na permeability ratio was found to be near unity and K:Na was about 0.7, both similar to the estimates obtained from 1 mM Ca experiments. The fact that the additional 55 mM Na caused rather little slow secondary increase in current after the initial jump also suggested that the conductance was near its maximum in the 55 mM Na, 1 μM Ca solution.

In the same manner Rb and Cs were found to go through the light-sensitive conductance (data not shown). The permeability ratio Rb:Na was ~0.4–0.5, and Cs:Na was ~0·2–0.3. In addition, Rb had an inhibitory action like K. Since the relative selectivity for monovalent cations seems to stay unchanged in normal and low Ca conditions, low Ca perhaps simply increases the effective number or fractional open time of the conductance sites.

We have also found that the divalent cations Ba and Sr go through the light-sensitive conductance in both normal and low Ca solutions. A large light-sensitive Ca current was similarly detected when the rod outer segment was exposed to isotonic Ca immediately after the 55 mM Na, 1 μM Ca priming solution mentioned above[14], though for reasons still not well understood no Ca current could be observed when Na in normal Ringer's was completely replaced by Ca. Ba and Sr generally have permeation properties similar to Ca[20]; it therefore seems most likely that Ca does go through the conductance in normal Ringer's. In physiological conditions such a Ca current is perhaps small relative to the Na current because of the large difference in their extracellular concentrations (1 mM versus 110 mM). A small but steady Ca influx through the light-sensitive conductance could, however, provide a simple replenishment mechanism for internal Ca, which otherwise would be gradually depleted by extrusion to the cell exterior in the presence of light[9,10].

The above experiments support and extend earlier observations that when external Ca is extremely low (≤1 μM), cations other than Na can go through the light-sensitive conductance[13,14,18,21,22]. They also underscore the key role of the Na–Ca exchanger in maintaining the open conductance and explain why earlier experiments with slower solution changes did not detect substantial ion movements besides Na in normal Ca conditions[13]. The relative abilities of Na and K to go through the conductance as measured above, together with estimates

of their internal concentrations in rods[23], are consistent with the reversal potential for the rod light response being at 0 to +10 mV[15–17].

The rapid perfusion method was developed jointly with Professor Alan Hodgkin and Drs Peter McNaughton and Brian Nunn. They have independently obtained similar results on monovalent cations and have also provided comments on this paper. We also thank Drs Denis Baylor, Malcolm Brodwick, Lee Moore and John Russell for comments and discussions. The work was supported by US National Eye Institute and the Retina Research Foundation, Houston, Texas.

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Intracellular Na^+ activates a K^+ channel in mammalian cardiac cells

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In a wide variety of cells, various intracellular agents, such as Ca^{2+} (refs 1, 2), ATP[3,4] and cyclic nucleotides[5,6], regulate ionic conductances of the membrane. In cardiac cells, the intracellular Na^+ concentration ($[Na^+]_i$) frequently increases when a disturbance occurs in the electrogenic Na–K pump activity or the Na–Ca exchange mechanism. We have investigated a possible role of $[Na^+]_i$ in controlling ion channels by using a patch-clamp method[7], and have found a K^+ channel that is gated by $[Na^+]_i$ >20 mM, but not by the intracellular Ca^{2+} concentration ($\sim 10^{-4}$ M). We report here that the channel has a unitary conductance of 207 ± 19 pS($n = 16$) with K^+ concentrations of 150 mM outside and 49 mM inside, and shows no detectable voltage-dependent kinetics. The Na^+-activated K^+ channel represents a novel class of ionic channel.

In our experiments we used guinea pig ventricular single myocytes, dissociated by treatment with collagenase[8,9] (0.04%; Sigma type I). In the inside-out patch clamp experiments, at least three types of single-channel activities were observed with a pipette solution containing 150 mM KCl. One type of channel was identified as the inward rectifier K^+ channel[10–12] as it had a slope conductance (γ) of 32 pS, a reversal potential (25–30 mV) near the K^+ equilibrium potential (E_K) of 29 mV, and a mean open time of 200–250 ms at −40 mV (Fig. 1*ai*). The opening probability of the channel decreased with hyperpolarization. For the second type of channel, γ was 94 pS (Fig. 1*aii*) and there was little voltage dependency in the opening probability. The reversal potential of this channel was 30 mV, which was also close to E_K. The channel opening was blocked by 2 mM ATP inside the membrane. Thus, this channel is identical to the ATP-sensitive K^+ channel reported previously[4]. The third type of channel activity (Fig. 1*aiii*), however, has not been described previously; the γ for this channel was 220 pS (range 179–240 pS) in the potential range −60 mV to 0 mV. The reversal potential was 28 mV (Fig. 1*b*; mean = 28.7 ± 5.5 mV, $n = 12$), which is very close to E_K. With 50 mM K^+ and 100 mM Na^+ in the pipette, γ was 77 pS ($n = 3$) and the reversal potential was 1 mV, suggesting a 58 mV change for a 10-fold change in external K^+ concentration. Therefore, we conclude that this channel is selective for K^+. The slope conductance decreased at potentials more positive than 20 mV with 150 mM K^+ in the pipette (Fig. 1*b*), indicating an inward rectification of the single-channel current. Opening of the channel was usually accompanied by closures of short duration, which yielded bursts of openings (Fig. 1*aiii*). With 100 mM Na^+ on the inner side of the membrane, long intervals between bursts occurred only rarely. Therefore, the mean open and closed times were obtained from the arithmetical mean of the open or closed times. The mean open and closed times were 8.4 ± 2.0 ms and 6.5 ± 3.5 ms ($n = 4$), respectively, at 0 mV. The opening of the channel was unaffected by 2 mM ATP on the inner surface of the membrane. The probability of opening (P) was 0.49–0.54 and showed little voltage dependency at potentials between −40 mV and +20 mV (Fig. 1*c*). Thus, this type of K^+ channel differs from both the inward rectifier channel and the ATP-sensitive K^+ channel in single-channel conductance, mean open time and ATP sensitivity.

The value of P was found to depend on $[Na^+]_i$. Figure 2*a* shows that the activity of the channel was almost negligible in Na^+-free bathing solution (substituted by $Tris^+$); activity appeared only after the bath was perfused with 50 mM Na^+. We obtained similar results in eight other patches using $Tris^+$ or $choline^+$ as a Na^+ substitute. In contrast, $[Ca^{2+}]_i$ of 10^{-4} M failed to activate the channel (Fig. 2*b*). In this patch, we observed two channels after activation with 100 mM Na^+. Even when the channel was activated by Na^+, increasing $[Ca^{2+}]_i$ to 10^{-5} M had no significant effect on P. The inability of Ca^{2+} to activate the channel was confirmed in three other patches. Figure 3 shows the dose–response relationship of $[Na^+]_i$ to P for five patches. The channel was activated at about 20 mM, and P was 0.55 ± 0.10 ($n = 7$) at 100 mM $[Na^+]_i$. For a kinetic analysis, we considered, as a first approximation, a kinetic scheme having one open state and two closed states:

$$\text{open} \underset{\beta_1}{\overset{\alpha_1}{\rightleftharpoons}} \text{closed} \underset{\beta_2}{\overset{\alpha_2}{\rightleftharpoons}} \text{closed}$$

The transition rate constants (s^{-1}), calculated from the exponential distributions of the open and closed times[10] for a particular case with 50 mM K^+ in the pipette at −40 mV, were: $\alpha_1 = 422$, $\beta_1 = 398$, $\alpha_2 = 14.2$ and $\beta_2 = 63.1$ ($t_o = 2.51$ ms, $t_f = 2.05$ ms and $t_s = 81.3$ ms) with 30 mM Na^+ inside, and $\alpha_1 = 518$, $\beta_1 = 235$, $\alpha_2 = 44.3$ and $\beta_2 = 82.4$ ($t_o = 4.24$ ms, $t_f = 1.64$ ms and $t_s = 26.4$ ms) with 50 mM Na^+, where t_o, t_f and t_s are time constants of the open time distribution, and the fast and slow components of the closed time distribution, respectively. Thus, intracellular Na^+ affects all the rate constants. Among these effects, the predominant one might be an increase of α_2, which corresponds to a decrease in the interval between bursts. Within the pH range 8.0–6.5, P did not change significantly ($n = 3$). Li^+ (100 mM) could not substitute for Na^+ in the activation of the channel.

Further evidence for the activation of K^+ conductance by $[Na^+]_i$ was obtained in whole-cell clamp experiments. In Fig. 4,

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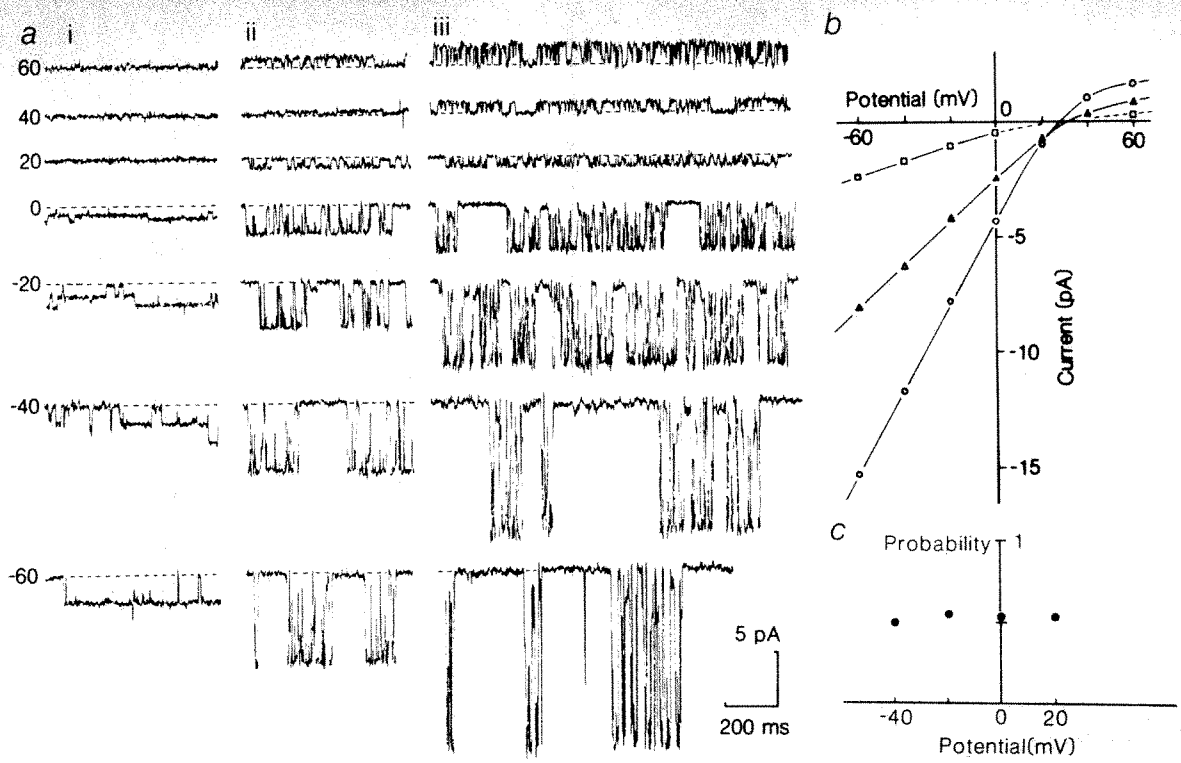


Fig. 1 Single-channel currents of three types of K^+ channel in inside-out patches. *a*, Current traces (recorded at potentials between −60 mV +60 mV) of the inward rectifier channel (i), ATP-sensitive channel (ii) and Na^+-activated K^+ channel (iii). *b*, Current–voltage relationship of the channels in *a*i (□), *a*ii (△) and *a*iii (○). The conductances measured at the linear part of the inward current were 32, 94 and 220 pS, respectively. *c*, Opening probability of the Na^+-activated channel in a different patch measured by integrating the current for at least 16 s. The compositions of the pipette and bath solutions were the same as for *a*iii. The pipette solution consisted of (in mM): KCl, 150; $CaCl_2$, 1.8; HEPES buffer, 5.0, *p*H 7.4. The bath solution for *a*i, *a*iii and *c* contained (in mM): KCl, 40; Na aspartate, 100; $MgCl_2$, 0.5; KH_2PO_4, 5.0; EGTA, 1.0; HEPES buffer, 5.0; glucose, 5.5; K_2-ATP, 2.0, with the *p*H adjusted to 7.4 by adding aspartic acid. In the patch illustrated in *a*ii, ATP was 0.5 mM; for the others the same concentration as in *a*i was used. Temperature of the perfusate was kept at 30–35 °C.

the inside of the cell was first dialysed with a Na^+-free solution, while perfusing the bath with Ca^{2+}-free Tyrode's solution. When the pipette solution was changed to 45 mM Na^+ solution, there was a shift in the current–voltage curve consistent with the activation of an outward current. The reversal potential of the activated conductance was −66, −67, −73 and −75 mV in four trials with three cells, consistent with E_K of −74 mV. The current induced by intracellular Na^+ shows an inward rectification around its reversal potential, but outwardly rectifies at potentials more positive than −24 mV. It is not clear whether this is due to a property of the Na^+-activated channel or an activation of other conductance systems.

These results indicate that intracellular Na^+ regulates the gating of a specific type of K^+ channel. The Na^+-activated K^+ channel can be characterized by its large conductance (207 pS), its inward rectifying property and its lack of appreciable voltage dependence of *P*. K^+ channels of very high conductance (~240 pS) have been reported in several tissues[13], most of which

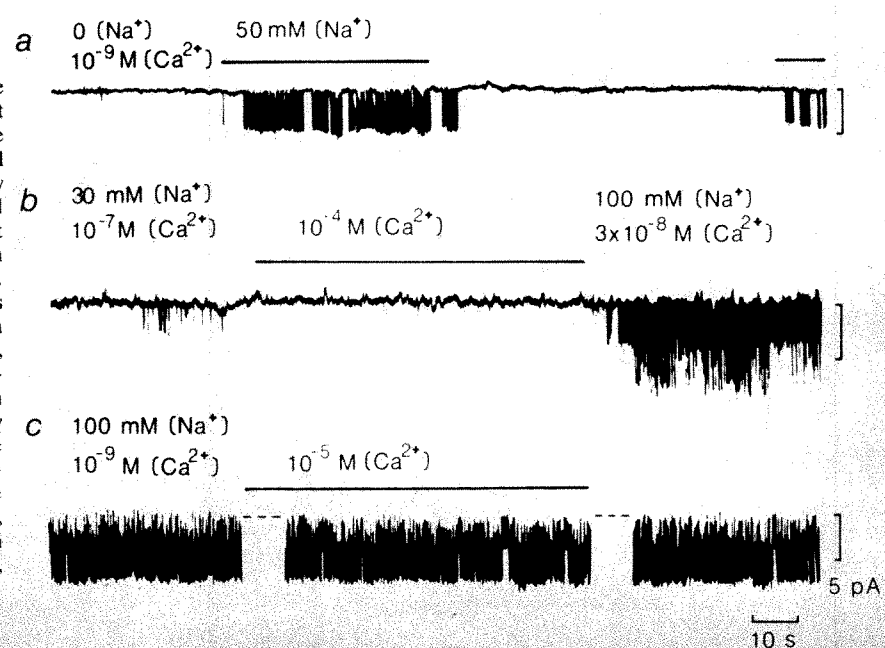


Fig. 2 *a*, Activating effect of $[Na^+]_i$ on the channel. Bath solutions with and without 50 mM Na^+ (0 $[Na^+]$ substituted by $Tris^+$) were alternatively perfused. Membrane potential was held at −30 mV. The opening probability (*P*) in 50 mM Na^+ was 0.54 and slightly reduced at the second trial. *b*, Lack of activating effect of $[Ca^{2+}]_i$ on the channel. The bath solution contained 30 mM Na^+ and 70 mM $Tris^+$. $[Ca^{2+}]_i$, buffered with Ca-EGTA buffer, was raised from 10^{-7} to 10^{-4} M, as calibrated by a Ca^{2+}-sensitive electrode (Orion Res. Inc., Cambridge, USA). Two channels were activated in the same patch by 100 mM Na^+, in which *P* was initially 0.23 and gradually increased to 0.48. *c*, Effect of $[Ca^{2+}]_i$ on the channel activated by 100 mM Na^+. Interruptions of the trace, lasting 3 min, represent the change of $[Ca^{2+}]_i$. The values of *P* before, during and after the elevation of $[Ca^{2+}]_i$ from 10^{-9} to 10^{-5} M were 0.60, 0.57 and 0.59, respectively.

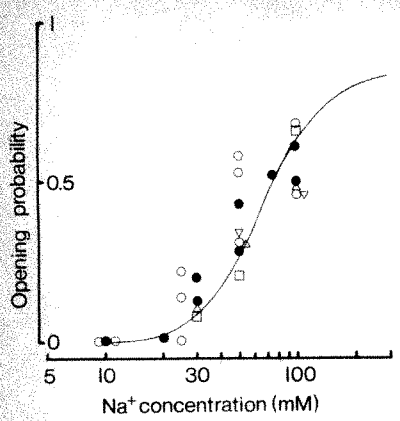


Fig. 3 Dose–response relationship between $[Na^+]_i$ and the opening probability of the channel. Measurements were performed in five different patches as indicated by the different symbols. A theoretical curve was drawn according to the following equation: $P = 0.85/1 + (K_D/Na_i)^n$, where K_D is a dissociation constant and n is Hill's coefficient. Using a least-squares fit, the values of K_D and n were found to be 66 mM and 2.8, respectively. The data indicated by solid circles were obtained in an opened cell-attached patch, where a part of the cell membrane was chemically skinned by treatment with saponin (10 mg ml^{-1}) while keeping the cell-attached patch intact.

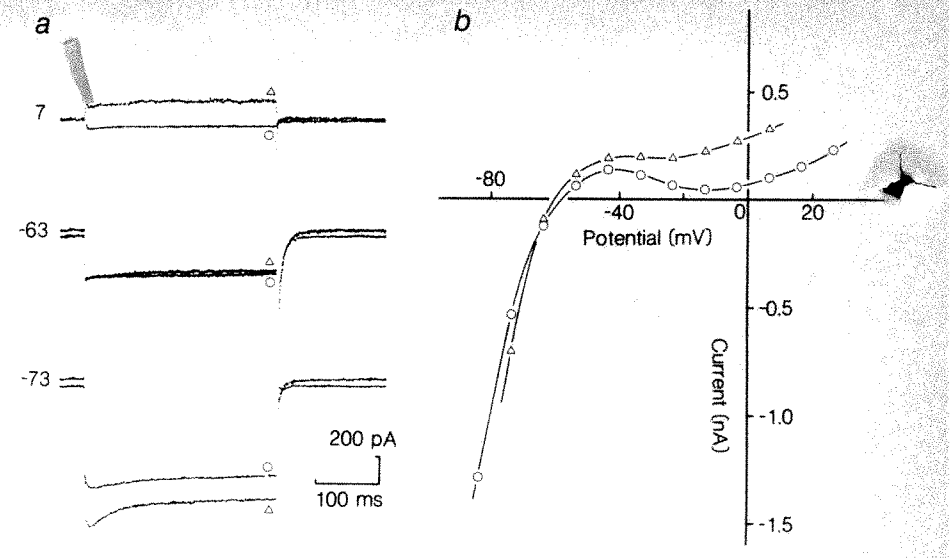


Fig. 4 Activation of a K^+ conductance by an increase in $[Na^+]_i$ in the whole-cell clamp. *a*, Current traces recorded with Na^+-free internal solution (○) containing (in mM): K-aspartate, 90; Tris-HCl, 50; K_2-ATP, 5; EGTA, 5, *p*H 7.4. Thereafter, 45 mM Tris-HCl was replaced with equimolar NaCl (△). The bath solution contained (in mM): NaCl, 143; KCl, 5.4; $MgCl_2$, 0.53; NaH_2PO_4, 0.33; glucose, 5.5; HEPES buffer, 5.0, *p*H 7.4. The holding potential was set at −43 mV to inactivate the Na^+ current. Test potentials are indicated at the left of each trace. *b*, Current–voltage relationship for the cell in *a*. The slope conductance at −67 mV was increased by 36%.

are Ca^{2+}-activated K^+ channels. It is of interest that the inward rectifier current in starfish egg cells requires intracellular Na^+ to be activated by hyperpolarization[14]. A similar mechanism might underlie the interaction of Na^+ with the channel reported here.

In normal cardiac cells, $[Na^+]_i$ has been reported to be <10 mM (refs 15, 16), a concentration which is balanced between the influx through Na^+ channels, the Na–Ca exchange mechanism and other passive leakage pathways, and active extrusion via the Na–K pump[17] as in other excitable cells. A prolonged failure of Na^+ pumping increases $[Na^+]_i$, resulting in a less efficient Ca^{2+} extrusion by the Na–Ca exchange, or even in a reversal of this mechanism due to a decrease in the Na^+ driving force[18,19]. Activation of K^+ conductance by the elevated $[Na^+]_i$ might improve the Ca^{2+} transport by opposing depolarization of the cells.

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Adoptive transfer of myelin basic protein-sensitized T cells produces chronic relapsing demyelinating disease in mice

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The autoimmune disease of the central nervous system (CNS), experimental allergic encephalomyelitis (EAE), is induced by challenge of genetically susceptible animals with spinal cord homogenates or myelin basic protein (MBP)[1–4]. Chronic and relapsing forms of the disease have some similarities to human demyelinating disorders, namely, multiple sclerosis[3,5–7], and are of particular interest. EAE can be transferred passively with sensitized lymphoid cells into syngeneic animals[8] but transferred EAE has been believed to have limited relevance to human disease because it is usually monophasic and manifested by minimal demyelination[9]. We report here that a single transfer of MBP-sensitized lymph node cells or T cells, in the absence of a peripheral antigen depot, leads to both acute EAE with significant primary demyelination, and chronic relapsing disease with lesions typical of demyelination over a long period. These findings have major implications for the immunological mechanisms involved in experimental and human demyelinating diseases.

Guinea pig myelin basic protein (GPBP) was prepared by the method of Diebler *et al.*[10]. The purity of the preparation was established by polyacrylamide gel electrophoresis. Female SJL/J

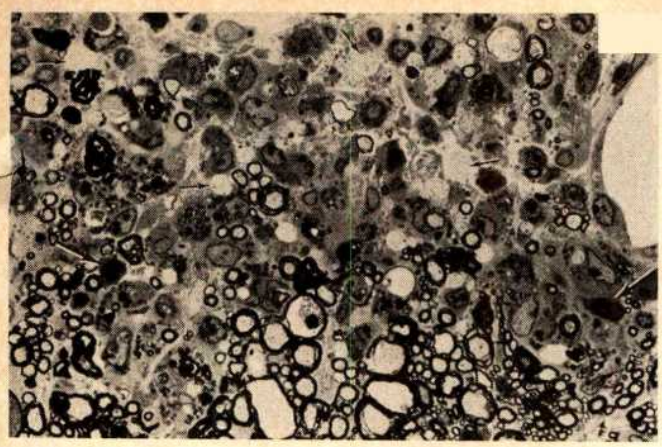


Fig. 1 Acute passively transferred EAE, 14 days post-transfer (1 μm epoxy sections stained with toluidine blue). Thoracic spinal cord. Higher magnification shows demyelinated axons (small arrows), large mononuclear cells containing myelin debris and at the edge of the lesion, polymorphonuclear leukocytes (large arrows). Some axonal damage and unaffected fibres are seen below. ×600.

mice, 6–10 weeks old, were injected subcutaneously at four sites, with 400 μg GPBP and 30 μg *Mycobacterium tuberculosis* in complete Freund's adjuvant[11]. Ten days later, the animals were killed; the draining lymph nodes were removed and passed through mesh in Hank's balanced salt solution (HBSS) to obtain a single cell suspension. Total lymph node cell (LNC) suspensions or B-cell-depleted suspensions, prepared by panning on anti-mouse IgG coated plates[12], were adjusted to 4×10^6 per ml and cultured in the presence of GPBP[11]. Four days later, the cells were removed and washed extensively. B-cell-depleted LNC were depleted further of adherent cells[13] by two successive incubations on Petri dishes precoated with fetal calf serum for 90 min at 37 °C. The desired numbers of LNC or T cells in 0.2 ml were inoculated into the tail vein of syngeneic recipients.

Animals were observed daily for clinical signs of EAE. The severity of the disease was graded on a scale of 1–5 (ref. 11). Relapses were only scored when a mouse developed a similar or more severe neurological deficit following a previous improvement. Routine histology was performed as described previously[4]. Selected animals between 14 days and 3 months after inoculation were perfused with 20 ml of 2.5% glutaraldehyde in PO_4 buffer and post-fixed in 1% osmium tetroxide. Blocks were dehydrated in ethyl alcohol and embedded in Epon 812. Epoxy sections 1 μm thick were stained with toluidine blue[14].

All recipients of $2–6\times10^7$ LNC or T cells developed clinical and histological signs of acute EAE 7–10 days after inoculation (Table 1, expts 1, 2). A few recipients of 7×10^6 cells showed a delayed onset of clinical disease as late as 82 days after transfer.

Approximately 20% of mice receiving 6×10^7 LNC died. After 2 weeks, the remaining mice with acute EAE either stabilized clinically, or gradually recovered with residual signs. At this time, some mice were killed for pathological examination and others were maintained for long-term clinical observations. Morphologically, the CNS of mice with acute disease showed perivascular inflammatory infiltrates in the meninges and parenchyma. The latter contained large lesions consisting of a primary wave of polymorphonuclear leukocytes which was followed by mononuclear cells. There was widespread loss of myelin but remarkable sparing of axons throughout the CNS, particularly in the subpial portion of spinal cord (Fig. 1); axonal degeneration was occasionally observed.

In five consecutive experiments, every mouse which recovered after the acute attack developed chronic relapsing disease. This usually began 30 days after the initial attack. After each relapse, the animals' conditions improved, but never returned to normal and with time there was progressive deterioration. Two representative experiments are shown in Table 1. In all studies, the potency of the adoptive transfer of chronic relapsing EAE, as judged by the number of relapses, the interval between relapses, clinical severity at the end of each attack and pathology was proportional to the number of LNC or T cells transferred.

Mice with chronic disease showed large lesions (Fig. 2) which were gliotic and contained inflammatory infiltrates consisting of a few polymorphonuclear leukocytes, many lymphocytes and macrophages. Although some axonal depletion was found, primary demyelination with sparing of axons was always evident and CNS remyelination was common (Fig. 3). The optic nerves were severely affected and dense collections of inflammatory cells were found within the angus of the lateral ventricles.

Chronic relapsing EAE has been induced in guinea pigs[2,3] and mice[4] by subcutaneous inoculation of CNS homogenates. Immunization with MBP may lead to relapsing disease in

Table 1 Adoptive transfer of chronic relapsing EAE in SJL/J mice by LNC primed *in vivo* and cultured *in vitro* with GPBP

Type and no. of cells transferred $\times10^{-7}$	Day of first attack	Clinical stage at first attack	No. relapses and day after transfer each occurred	Clinical stages at each relapse
Expt 1*				
LNC				
6	6	3	5(42, 69, 119, 142, 168)	3, 4, 4, 5, 5
6	7	4	5(41, 72, 114, 139, 170)	3, 3, 4, 4, 5
6	7	4	4(39, 75, 118, 152b)	4, 3, 4, 5
6	6	4	1(38, died on 58)	
2	10	4	2(49, 95)†	4, 4
2	10	3	2(51, 92)†	3, 4
2	11	2	4(54, 84, 110, 159)	3, 3, 4, 4
2	11	2	3(51, 103, 153)	3, 4, 4
0.7	10	2	2(110, 159)	3, 3
0.7	10	2	2(116–165)	2, 3
Expt 2‡				
T cells				
2	10	3	3(27, 58, 89)	4, 4, 5
2	10	4	3(31, 60, 91)	2, 3, 5
2	10	4	3(35, 55, 88)	3, 4, 5
2	10	4	3(39, 58, 85)	1, 2, 3
0.7	10	2	2(64, 93)	1, 2
0.7	10	2	2(70, 95)	2, 2

* Observation period was 180 days for this experiment.

† Mice were killed for histological study.

‡ LNC were depleted of B cells before the culture and of macrophages after the 4-day culture. The observation period for this experiment was 95 days.

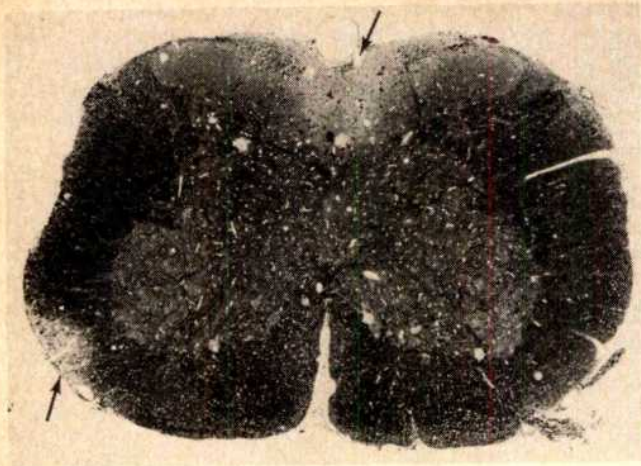


Fig. 2 Chronic relapsing EAE, 3 months post-transfer. Large, demyelinated gliotic lesions are seen in the dorsal and antero-lateral columns (arrows). Upper cervical spinal cord. ×35.

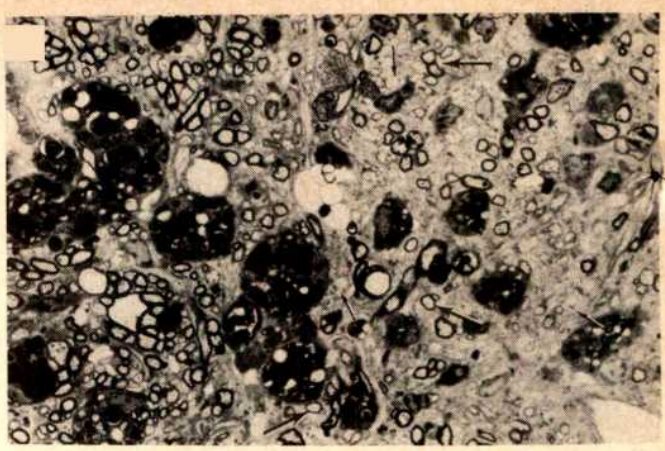


Fig. 3 Chronic relapsing EAE, 3 months post-transfer. At the edge of a chronic lesion, demyelinated axons (small arrows) and remyelinated axons (large arrows) can be seen. Towards the more normal white matter to the left, myelin degradation by large macrophages is apparent. ×600.

rats[15,16] and mice[17]. Injection of galactocerebroside with MBP augmented the level of demyelination observed in guinea pigs[18], and contamination of MBP preparations with lipid components has been reported[19]. These observations raise the possibility that trace amounts of lipids in our antigen preparation could contribute to the recurrent disease. Although this cannot be excluded, we believe it is unlikely because MBP prepared by the method used in this study does not produce demyelinating disease in guinea pigs.

Only acute EAE has been transferred to naive recipients either by sensitized lymphoid cells[8,11-16,20-23] or by T-cell lines[24]. Previously it was shown that the cells responsible for the transfer of acute murine EAE are Lyt-1$^+$2$^-$ T cells[11]. Recent work suggests that this subset of lymphocytes recognizes antigen in conjunction with class II molecules of the major histocompatibility complex (MHC). Although the reactive cells are directed at MBP, it is believed mostly to be localized in the major dense line of the myelin membrane[25] and therefore inaccessible to normal immune surveillance. Thus, reaction between MBP and immune cells may occur at some site other than the myelin membrane. MBP released during myelin turnover may be processed and presented to reactive cells. Indeed, macrophages[26], endothelial cells within the CNS[26] and oligodendroglia[27] have been reported to bear class II MHC molecules and could present antigen to immune cells. MBP is detectable in the blood of most species[28] which may help maintain immunological tolerance[29]. If MBP enters the circulation via the vascular endothelial cells, the luminal surface of such cells may be a site for interaction between immune cells and MBP[30]. This could lead to inflammation and result in subsequent myelin breakdown through a by-stander effect[28,31].

The mechanisms responsible for relapsing demyelinating disease in the absence of injected antigen are not known. One possibility is that MBP in antigen presenting cells is transferred along with the immune T cells and stimulates immune reactivity already present, but our previous findings with radiolabelled MBP indicate that the amount transferred would be less than 50 ng per animal[11]. Further, T cells depleted of antigen presenting cells still produced the chronic disease (Table 1, expt 2). Another possibility is that the acute disease produced by transferred T lymphocytes causes release of myelin antigen(s). MBP released with other myelin components during tissue damage may be more immunogenic than soluble MBP, and lead to alternation between the diseased and tolerant states[29]. Such antigen could either induce an immune response in the recipient or lead to an expansion of transferred memory effector cells. Rats immunized with cells from effector T-cell lines obtained from MBP-primed animals developed acute EAE but recovered, although they continued to harbour anti-MBP memory T cells in the thymus for months after the cell transfer[32]. Finally it is also possible that the acute disease induces an immune response against components of myelin other than MBP. Identification of the pathogenetic mechanisms responsible for the chronic autoimmune disease are of considerable importance because similar processes could occur in human demyelinating disorders.

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Phagocytosing macrophages exclude proteins from the zones of contact with opsonized targets

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During receptor-mediated phagocytosis, macrophages release toxic molecules such as hydrogen peroxide which enable them[1] to kill antibody-coated tumour cells[2] and parasites[3], too large to consume. Previous workers observed that while peroxide was clearly responsible for cytolysis of certain antibody-coated tumour cells[2,4,5], extracellular catalase was unable to inhibit this cytolysis, and they suggested that macrophages secrete peroxide into a protected cleft between the phagocyte and target. We have tested this and report here that the space beneath macrophages spread on glass surfaces is accessible to proteins with a molecular weight (MW) as large as 200,000 but the space beneath macrophages plated on glass surfaces coated with phagocytosis-promoting ligands is impermeable to proteins as small as 50,000 MW. It appears indeed that macrophages form a protein-tight seal at the periphery of their contact with ligand-coated surfaces and thereby create a closed compartment between the cell and the target.

To examine the ability of soluble proteins to permeate the zone of contact between a macrophage and a ligand-coated target, glass slides were coated with dinitrophenol (DNP)[6] and then with a monoclonal IgM anti-DNP antibody to give a IgM-coated surface (Fig. 1). IgM is not recognized by receptors on the macrophage, and therefore they spread on these surfaces as they would on glass or plastic. The purpose of the IgM-coated surfaces to provide binding sites for fluoresceinated anti-IgM antibodies. In parallel experiments, we incubated the DNP surfaces with a mixture of IgG–anti-DNP and IgM–anti-DNP (Fig. 1). The Fc domain of IgG is recognized by Fc receptors on macrophages, thus when the cells settle on such IgG-bearing surfaces they produce hydrogen peroxide[1] and spread extensively as though in an attempt to phagocytose the slide.

Human monocytes cultured for 4–12 days in Teflon beakers[7] were allowed to adhere to one of these ligand-coated surfaces. They were then incubated at 20 °C for 1.5 h with fluoresceinated anti-IgM, washed, fixed, and viewed with a fluorescence microscope. This antibody binds uniformly to IgM-coated substrates, even directly under the macrophage (Fig. 2*c*) and indicates that the space between the undersurface of adherent macrophages and the substrate is accessible to soluble proteins. In contrast, on IgG + IgM-coated surfaces, the fluoresceinated anti-IgM is excluded from the zone between the phagocyte and the substrate leaving 'black holes' in the fluorescent surface that correspond closely to the margins of the spread macrophage (Fig. 2*a*). This suggests that during phagocytosis, macrophages make a tight seal against the target surface that excludes soluble protein.

We have done several experiments to eliminate alternative explanations of these observations. The exclusion of fluoresceinated anti-IgM from the space beneath a phagocyte on an IgG-coated surface is not a consequence of the greater spreading or the evolution of peroxide seen when macrophages interact with IgG. If cells are treated with the tumour promoter, phorbol myristate acetate (PMA), the cells spread extensively and evolve large amounts of peroxide[1,8]. However, PMA-treated cells do not exclude fluoresceinated anti-IgM from their regions of contact with control IgM-coated surfaces (data not shown). Further, neither phagocytes nor their secretion products quench the antibody fluorescence because, when fluoresceinated anti-IgM is added to the IgG + IgM-coated surface before the cells are plated, the surface remains uniformly fluorescent. Finally, the cells do not consume or destroy the antigens recognized by the fluorescent antibody. When cells that had formed black holes are permeabilized with 1% Triton X-100 (in phosphate-buffered saline, 10 min, 5 °C), the IgM underlying the former holes can be stained with fluorescent antibody (data not shown).

We have confirmed our observations using electron microscopy. For these studies, we used horseradish peroxidase-labelled IgG anti-IgM (HRP anti-IgM) instead of fluorescent antibody, and the HRP anti-IgM was visualized by staining with diaminobenzidine. Sections cut perpendicular to the substrate show that HRP anti-IgM readily penetrates beneath macrophages spread on IgM-coated surfaces (Fig. 3*a*), but HRP anti-IgM cannot penetrate beneath macrophages engaged in a 'phagocytic' interaction with IgG + IgM-coated surfaces (Fig. 3*b*). The penetration of HRP anti-IgM stops within 0.28 (±0.15) μm of the margin of the cell, in a zone where the plasma membrane appears tightly adherent to the substrate. The entire plasma membrane is not, however, tightly adhered to IgG + IgM-coated surfaces. The membrane underlying the central area of the macrophage resembles that of a macrophage spread on a control substrate with interspersed regions of close apposition and regions well separated from the substrate (Fig. 3). Thus, there exists an open space or compartment between the cell membrane and substrate that is inaccessible to external proteins.

The exclusion of protein from the macrophage–substrate interface is not a simple physical consequence of receptor–ligand bridging. Macrophages have receptors for the third component of complement, C3, that mediate binding but not phagocytosis

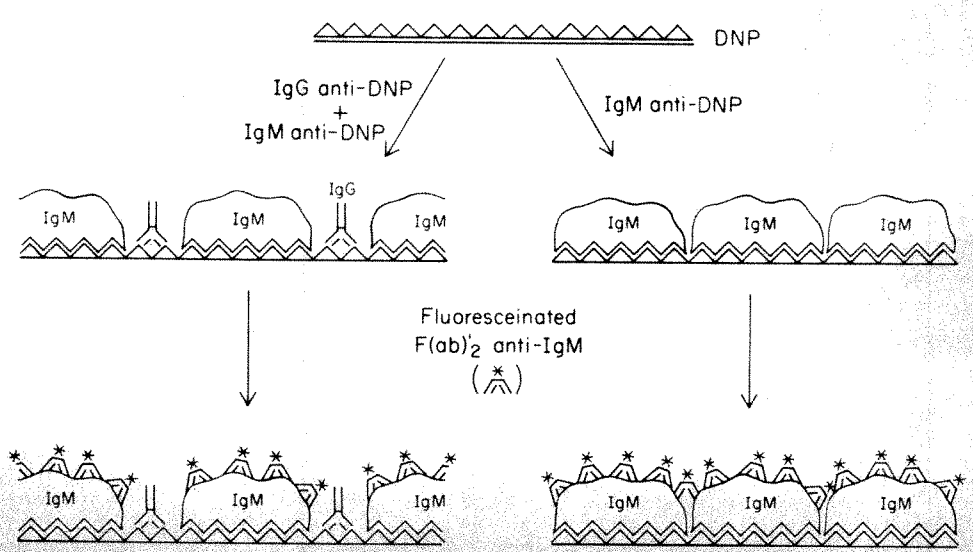


Fig. 1 Construction of ligand-coated surfaces. Masked slides (Carlson Scientific) were treated with poly-L-lysine then with 2,4-dinitrobenzene sulphonate to yield DNP-coated surfaces[6]. These surfaces were treated with 10 μl of murine monoclonal IgM anti-DNP (1:200 dilution of ascites fluid) with or without 10 μg ml^{-1} of affinity purified rabbit IgG anti-DNP (ref. 15) for 30 min at 20 °C. The surface was washed and macrophages were allowed to adhere to it. Fluoresceinated goat anti-IgM antibodies (IgG or the $F(ab')_2$, or Fab fragments (Cappell)) were diluted 1:100 in phosphate-buffered saline and added for 1.5 h at 20 °C. Fluoresceinated $F(ab')_2$ fragments are depicted here.

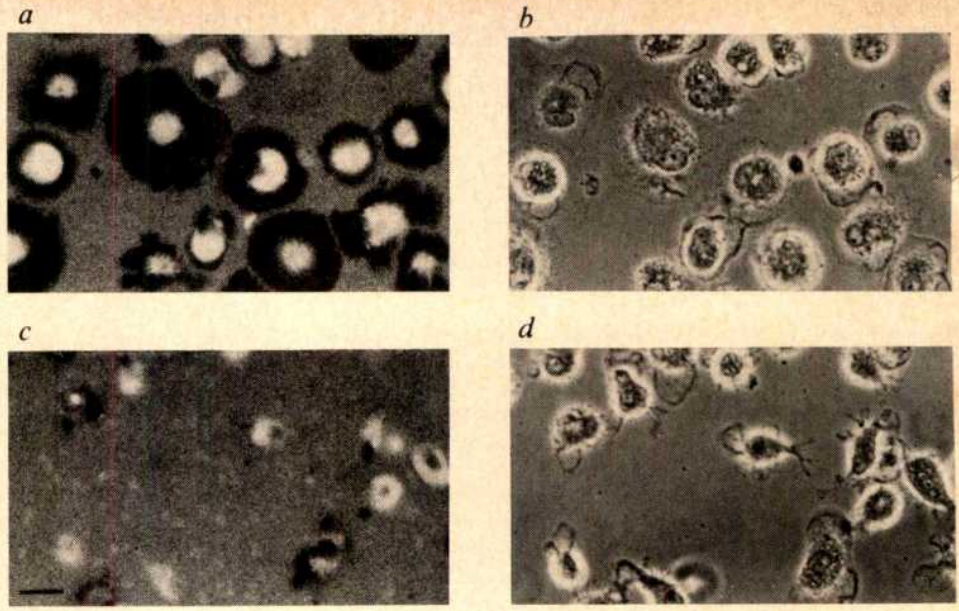


Fig. 2 Staining of ligand-coated substrates with fluoresceinated anti-IgM. Macrophages were cultured for 10 days in Teflon beakers, collected, then allowed to spread for 45 min at 37 °C on IgG + IgM-coated surfaces (*a*, *b*) or IgM-coated surfaces (*c*, *d*). The surfaces were then incubated for 1.5 h at 20 °C with a 1:100 dilution of fluoresceinated anti-IgM. Preparations were then washed, fixed in 3% formalin (diluted in phosphate-buffered saline) for 30 min at 20 °C, and photographed with a fluorescence microscope. The right-hand panels (*b*, *d*) are phase contrast photographs, and the left-hand panels (*a*, *c*) are fluorescent photographs of the same fields. Note that the IgM-coated surface underlying the cells is uniformly stained with the fluorescent antibody (*c*) but that cells spread on the IgG + IgM-coated surface protect the underlying antigens from binding the fluorescent anti-IgM (*a*). The centre of the cell appears bright owing to yellow autofluorescence that is easily distinguished from the green fluorescence of the antibody. In this experiment, fluoresceinated $F(ab')_2$ fragments of anti-IgM were used. Similar results were obtained using fluoresceinated Fab fragments or fluoresceinated whole IgG. Scale bar, 20 μm.

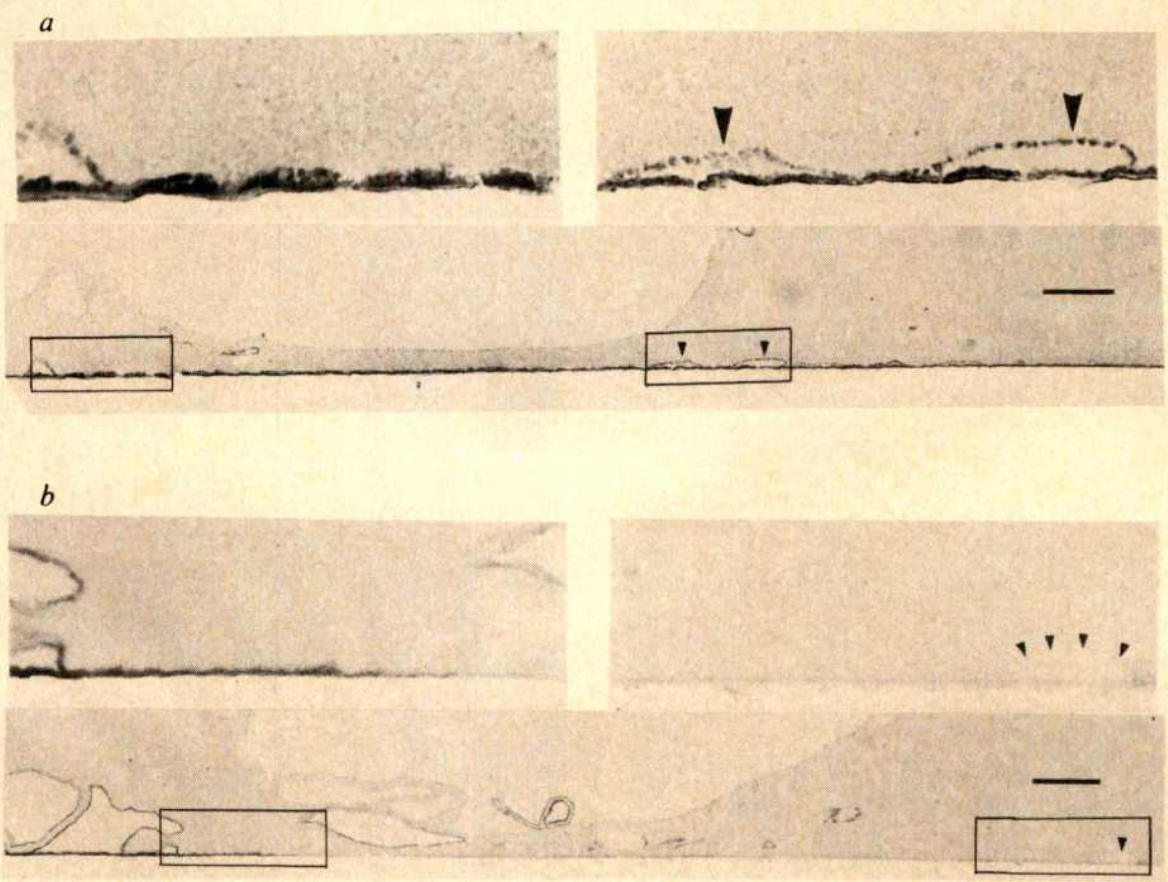


Fig. 3 Ultrastructural localization of horse radish peroxidase-labelled anti-IgM (HRP anti-IgM) on ligand-coated substrates. Macrophages were cultured for 8 days in Teflon beakers, collected, then allowed to spread for 45 min at 37 °C on IgM-coated surfaces (*a*) or IgG + IgM-coated surfaces (*b*). The surfaces were then incubated for 1.5 h at 20 °C with a 1:100 dilution of HRP anti-IgM, washed, then fixed for 1 h at 20 °C with 2.5% glutaraldehyde. HRP was stained using diaminobenzidine[16], the specimens were treated with 1% osmium tetroxide, and were then embedded in resin[17]. Sections cut perpendicular to the surface were viewed in an electron microscope without further staining. The electron-opaque reaction product generated by HRP is seen coating the entire IgM-coated surface, even directly under spread macrophages (*a*). In contrast, reaction product is not seen beneath cells spread on IgG + IgM-coated surfaces (*b*), even in locations where the plasma membrane rises from the substrate revealing an open space between the membrane and substrate (arrows). Boxed areas are shown in 3.5-fold enlargements. Scale bars, 0.1 μm.

of C3-coated erythrocytes[7]. We therefore constructed surfaces bearing C3 by incubating IgM-coated surfaces with a source of complement[9]. When macrophages spread on such surfaces C3 receptor activity is lost from the apical portion of the cell[7,10] because of diffusion of the receptors to the basal surface where they are 'trapped' by interaction with ligand[11]. However, despite this ligand–receptor interaction IgG anti-C3 can still penetrate readily beneath the macrophage. (To observe this, macrophages on C3-coated surfaces were cooled to 20 °C, then incubated for 45 min with murine IgG anti-C3, then for another 45 min with fluoresceinated Fab anti-IgG and examined by fluorescence microscopy.) In contrast, stimulated macrophages are able to phagocytose via their C3 receptors; the addition of 30 ng ml^{-1} PMA to macrophages cultures increases phagocytosis promoted by C3 receptors 10–50 fold[7]. Macrophages plated on a C3-coated surface in the presence of PMA exclude IgG anti-C3 from the interface with the substrate (data not shown). These experiments demonstrate that sealing of the contact zone between macrophage and target is not caused by receptor–ligand interaction alone but that cytoplasmic movements initiated by receptor–ligand interaction causes the sealing.

The spreading of cultured cells on glass or plastic has been likened to the cells' attempt to phagocytose a particle of infinite diameter. Here we show, however, that spreading and phagocytosis appear quite different. After spreading, the space between macrophage and substrate is freely accessible to proteins as large as 200,000 MW, but during a 'phagocytic' interaction with the substrate, proteins as small as 50,000 MW (Fab fragments of fluoresceinated anti-IgM) are excluded from the phagocyte–substrate interface. Thus it appears that the seal between macrophage and substrate is brought about by cellular movements characteristic of phagocytosis but not by the movements of spreading.

During phagocytosis, macrophages form the seal as a continuous rim at the leading edge of the advancing pseudopod, thus creating a closed compartment between phagocyte and target (Fig. 3). This observation is consistent with previous electron microscope studies which show the phagocyte's plasma membrane tightly appressed to the target at the leading edge and an open space between the macrophage and target at the base of the forming phagosome[11,12]. The presence of such a protected compartment has been predicted from experiments on the cytolytic action of phagocytes[2,4,5] and from studies of ligand-induced modulation of Fc receptors[13,14] and the data presented here provide evidence for its existence. We believe that this compartment is used to concentrate, protect and target toxic substances that may be specifically released into this space by the phagocyte.

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Separation of developmental compartments by a cell type with reduced junctional permeability

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Two recent reports[1,2] have suggested that the junctional membrane of the cells located at compartment boundaries in developing insect epithelia, although permeable to inorganic ions[3,4], prevents the cell-to-cell transfer of the fluorescent tracer Lucifer yellow (LY). However, cells within a compartment are linked by junctions permeable to both inorganic ions and organic tracers[1–5]. These findings imply that junctional communication is important in tissue development and that spatial selectivity may allow developmental autonomy in nearby compartments. The observation that LY was occasionally seen to pass across the segment border of the milkweed bug *Oncopeltus fasciatus*[1] suggested to us that the permeability of the junctions of the segment border may be locally controlled. We report here that the selectivity in the junctional channels at compartment boundaries in this insect is variable and under the control of a discrete population of border cells. The extent to which these cells reduce the rate of tracer passage across the compartment boundary relative to that within the compartment is influenced by the culture medium in which the tissue is placed.

Pieces of integument, containing three segment borders, were dissected from the dorsal abdominal wall of fourth instar nymphs of the mutant strain *cb;wb* (ref. 6). Epidermis of this stage was selected over that of the fifth instar studied previously[1] because the cuticle is smoother at the segment borders and allows greater cellular detail to be seen. The preparation was clamped in a culture dish containing one of three bathing media (Table 1), and examined at ×40 objective magnification by phase-epifluorescence[7]. Routine methods were used to microinject dye into cells by iontophoresis[8].

The transverse border of each segment is usually marked by the presence of several rows of elongated cells ('border cells') with their long axes parallel to the border[9]. The pigmentation and clonal origin of these cells suggest that they have a common lineage with the more posterior segment, that is, they are at the leading edge of the segment[9]. The border cells confront the most posterior cells of the segment ahead. Both these posterior cells and the general cells of the posterior segment are normal in shape (regular polygons) (Figs 1–3).

Three fluorescent dyes were used to probe the junctional permeability of the border cells: Lucifer yellow (LY, molecular weight 457), 6-carboxyfluorescein (CF, molecular weight 376), and lissamine rhodamine B (LRB, molecular weight 559). Dye was injected into a normally shaped cell one or two cells distant from the strip of border cells. The extent of dye spread was recorded photographically (Figs 1, 2), while the rate of dye passage in the cell sheet was determined from spreads videotaped during dye injection[10,11].

The cell-to-cell spread of all three dyes was radially asymmetrical (Fig. 1), as their passage into and across the strip of border cells was always retarded. In a typical LY spread (Fig. 1*a*), appreciable amounts of the dye passed into the cells next to the source cell within 3 s, and into the border cells within 10 s. A drop in fluorescence intensity coincided with the margins of the border cells. Within 30 s the dye had passed across the border into the normally shaped cells of the next segment, to image cell detail such as nuclei, vacuoles and cell margins. A dead cell in the recipient segment was silhouetted by the dye diffusing around it. This was observed irrespective of whether the source cell was anterior or posterior to the border. The 'texture' to the dye spread is proof that the dye passed from cell to cell and was not due to scattered light or to the dye passing between the cells and the underlying cuticle. The general

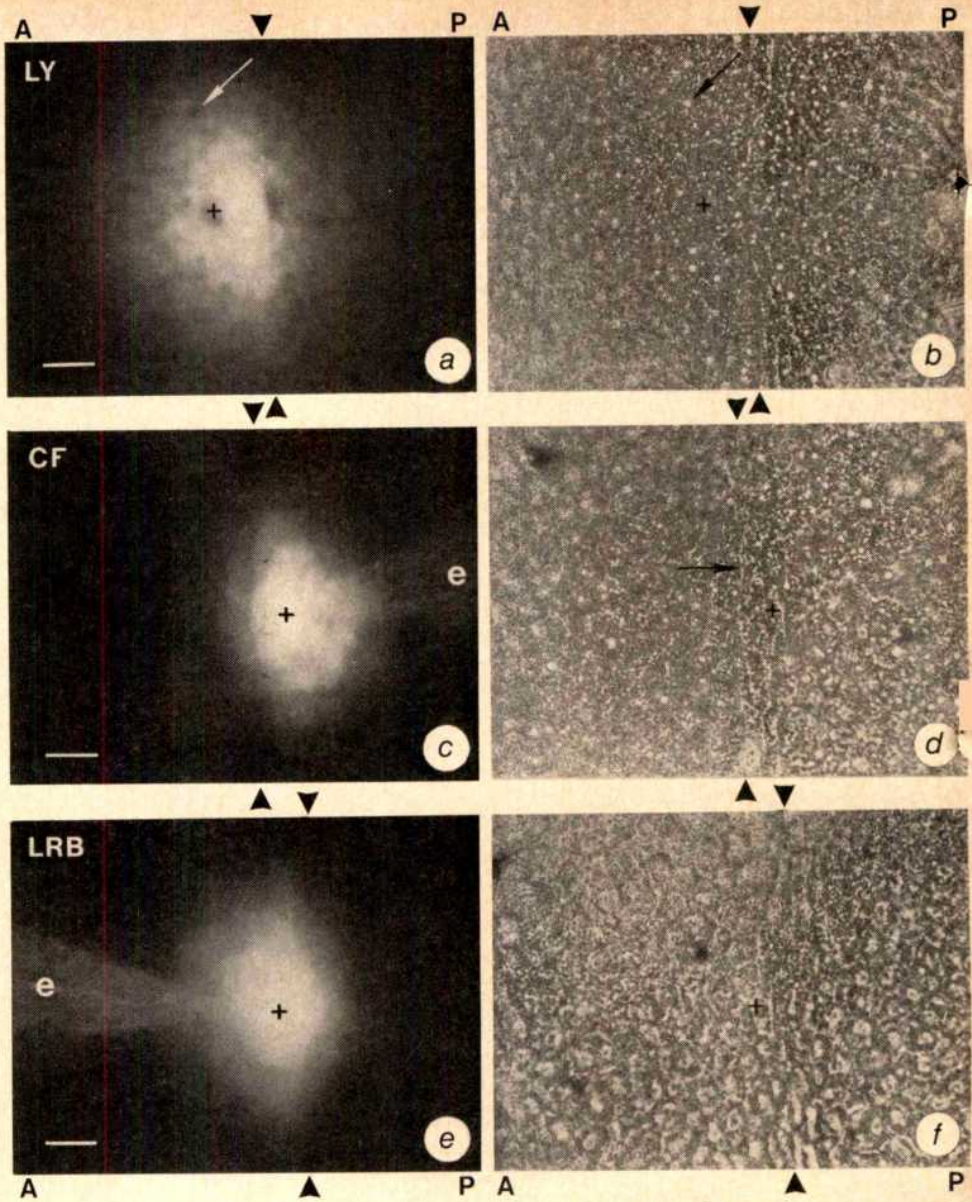


Fig. 1 Fluorescent tracers microinjected into epidermal cells near the segment border pass across it, although a drop in fluorescence occurs in this region. Left: record of three different fluorescent tracers spreading rapidly across the segment border. Right: the same preparations shown in phase contrast. The position of the border is marked by the arrowheads above and beneath the micrographs. A, anterior segment; P, posterior segment. Scale bar, 25 μm. *a*, Lucifer yellow (LY) was injected into a cell (+) anterior to the segment border for 30 s, the electrode was then removed and the extent of dye spread photographed immediately (60 s exposure). The spread of dye is impeded by the border. The appearance of the cells, imaged by dye localized in their nuclear regions, suggests that the border cells were not particularly elongated in this preparation. The source cell was damaged during electrode removal and failed to retain the dye. The dark area just across the border from the injection site is a dead cell imaged by the dye that diffused around it. *b*, Phase-contrast appearance of *a*. The most posterior cells of one segment are more opaque than the anterior cells of the adjacent segment. In some cells of preparations such as this one, the presence of small vesicles that exclude dye (diagonal arrow in *a* and *b*) allow the micrograph pairs to be matched up independently of the cell margins, which are often obscure. *c*, Carboxyfluorescein (CF) injected for 20 s, into a cell (+) posterior to the segment border, and the extent of its spread photographed (7 s exposure). CF has crossed the border within 30 s of the start of injection. This dye fills the individual cells more uniformly than does LY (*a*). The tip of the electrode (e) was left in the source cell after the period of dye injection was over. *d*, Phase-contrast appearance of *c*. In the centre of the micrograph the position of the segment boundary is marked by a thin intercellular space (horizontal arrow). Cell margins, although generally difficult to discern here, are seen as step-like drops in fluorescence in *c*. *e*, Lissamine rhodamine B (LRB) injected continuously for 120 s into a cell (+) in the anterior compartment and the spread photographed (12 s exposure) with the electrode (e) still inside the source cell. This dye was the slowest to image cell detail across the border. *f*, Phase-contrast appearance of *e*.

Methods: Cell preparations were excised from fourth instar nymphs in the middle of the instar, placed in medium 3 (ref. 15) and used within 60 min of dissection. Dye was iontophoresed from an electrode (200-ms current pulses, 1 per s; strength in *a*, 60 nA; in *c* and *e*, 6 nA) inserted into a normally shaped cell (the source cell) one or two cells distant from the border cell strip. Placing the electrode in this position prevented the tip from crossing the border accidentally, either during its insertion into the source cell or due to any slight electrode drift during injection. Both could give a false positive for the dye passage across the border. The source cell was close enough to the border to allow dye to bank up against it, and passage across it to be detected. The entry of the dye electrode into the source cell was timed from the moment its membrane potential was recorded on the chart recorder. Criteria for a successful dye spread were a stable membrane potential, between 26 and 35 mV during the period of dye injection, and the presence of cell detail in the fluorescence image.

features of the cell-to-cell passage of CF and LRB both within and between segments were the same as those for LY, although the rates differed. The spread of LRB was considerably slower than that of the other two dyes, as has been noted in another insect epidermis[11].

We noticed also that the rate of dye spread was considerably influenced by the medium in which the tissue was placed (Table 1). In some media, such as that used for the preparations shown in Fig. 1, the border offers little resistance to dye spread; in others, dye passage is almost totally blocked in the border region. This observation suggests that the cells at the segment border can regulate the segment-to-segment passage of cytoplasmic molecules, but does not indicate whether the asymmetrical spread of dye is the result of tighter packing of border cells (since dye spreads more rapidly within cells than between cells) or whether the permeability of the junctional channels in the border region is set at a different level from that elsewhere in the segment. To show that the altered shape of these cells is not the sole cause of the impeded dye spread, some preparations were placed in medium that depressed segment-to-segment dye coupling, a cell posterior to the border cells was injected with CF, and the extent of its spread photographed. The procedure was repeated on a cell anterior to the border, directly opposite the first cell. The result (Fig. 2) has two main features. First, dye spread is virtually blocked in the border region in both cases (in contrast to Fig. 1). It is unlikely that this could be due to changes in cell packing. Second, the lines along which the dye movement is restricted are not the same, but coincide instead

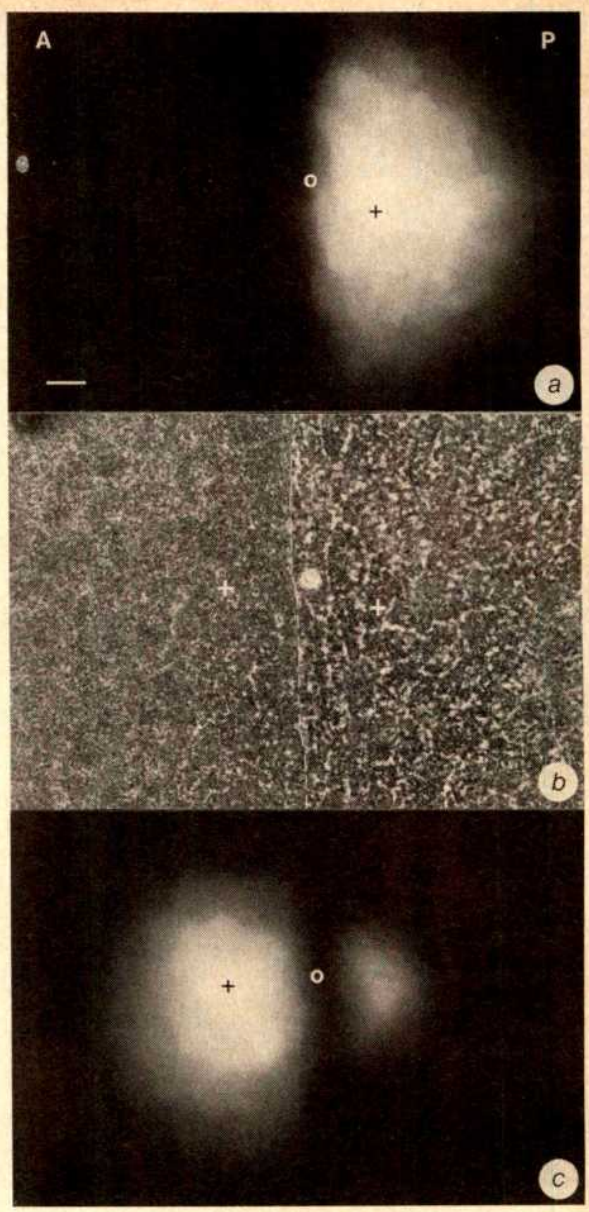


Fig. 2 When tracer was injected into a cell near the segment border in preparations immersed in medium 1 of Table 1, its spread was blocked along a line parallel to the segment border. Dye injected into the anterior segment was blocked at the border while that injected posterior to it was blocked at a line that did not coincide with the segment border, but was separated from it by a strip of cells. The junctional permeability of this border strip of cells appears to be regulated independently, since dye moved rapidly among cells within a segment. CF was injected (200-ms pulses of 60 nA, 1 per s) into cells labelled +. A, anterior segment; P, posterior segment. Scale bars, 10 μm. *a*, CF injected into a cell posterior to the segment border for 9 s and the extent of dye spread photographed 22 s later (4 s exposure) with the electrode removed. The dye spread in a radially asymmetrical fashion and was blocked in its passage along a line passing through a marker particle (white circle, see *b*). Contrast the shape of this spread with that in Fig. 1c, where the spread of dye is only slightly retarded in the border region. *b*, Phase-contrast view of the cells shown in *a* and *c*. The more opaque cells of the anterior segment are distinguishable from the clearer cells of the posterior segment; the position of the segment border is emphasized by the dashed white line. The refractile particle lying just to the right of the segment border serves as a reference for locating it in *a* and *c*. *c*, Dye injected for 8 s and its fluorescence photographed 23 s later (4 s exposure) with the electrode removed. Dye spread is asymmetrical with its movement strongly truncated at the segment border, just to the left of the marker particle (white circle). Residual fluoresence from the spread shown in *a* done 4.5 min earlier is seen to the right of the marker particle. A drop in tracer fluorescence at the border could be an artefact caused by a reduction in cell thickness or changes in the cuticle in this region. Histological sections, however, showed the integument to be flat and the cell and cuticle thickness to remain constant across the border in our fourth instar preparations. Since the fluorescence was viewed through the cuticle, it is also possible that a reduction in the light-transmitting properties of the cuticle might lower fluorescence at the border. Consequently, preparations injected with dye were then inverted and re-examined. With the cuticle removed from the light path, no change in the intensity and distribution of fluorescence was seen.

with the margin of the border cell population nearest the injection cell. In both cases, the dye spread one or two cell diameters towards the border before being stopped by the border cells, while at the same time spreading four to five cell diameters away from the border. Here, it appears that there is a separate population of border cells having greatly reduced junctional permeability.

The presence of a cell type with different junctional properties can also be demonstrated by injecting dye simultaneously into two cells, one on either side of the border (Fig. 3). A narrow strip of elongated cells, lying just posterior to the segment border, is revealed by its lower tracer permeability, despite a high concentration of dye potentially able to enter these cells from both sides. When a border cell identified in this way was subsequently injected with dye, it passed at best slowly and radially into the surrounding cells. No preferential movement of dye among the border cells was detected.

Although Warner and Lawrence[1] clearly demonstrated an alteration in the normal cell-to-cell diffusion of microinjected tracers at the compartment border, they did not explain how this was achieved. The data suggested, nevertheless, that reduced junctional permeability at the border results from the confrontation of the surfaces of cells belonging to different compartments. This implies that communication is reduced only at areas of membrane junctions connecting cells of adjacent compartments, but that it remains normal at junctions connecting border cells with other cells in the same compartment. However, this appears not to be the case. *In vitro*, reduced communication between compartments in the bug is due to the intervention of a discrete population of cells (located at the anterior margin of each segment) whose junctions in certain culture media all have reduced permeability. These cells communicate less not only with the cells of the compartment anterior to them, but also among themselves and with the general epidermis of their own

Table 1 Extent of cell-to-cell spread of three fluorescent tracers across the segment border

Tracer	No. of preparations tested	Appearance of individual dye spreads		
		a	*b*	*c*
Carboxyfluorescein	29	13 (1)	69 (1–3)	3 (3)
Lucifer yellow	10	0	25 (1–3)	8 (2, 3)
Lissamine rhodamine B	19	10 (1)	36 (1–3)	2 (3)

About equal numbers of spreads were prepared in which the source cell lay anterior or posterior to the border. No differences in dye spread were noted. *a*, Dye spread restricted to within one segment by border cells; *b*, dye spread into adjacent segment but impeded by border cells; *c*, dye spread minimally retarded by border cells. As we were concerned that the composition of the culture medium might influence junctional permeability, three media were tested. Each preparation was used in only one medium. Numbers in parentheses refer to the media in which a particular result was obtained. Medium 1 was TC 199 containing 10 mM PIPES buffer, medium 2 was leafhopper medium[14] and medium 3 was a *Drosophila* medium[15] lacking amino acids. The media contained 10% fetal calf serum and the *p*H was adjusted to 6.7 with NaOH. Of the three, medium 3 least retarded the movement of fluorescent tracer across the border. It also resembled most closely the blood of phytophagous hemiptera in its monovalent and divalent cation levels.

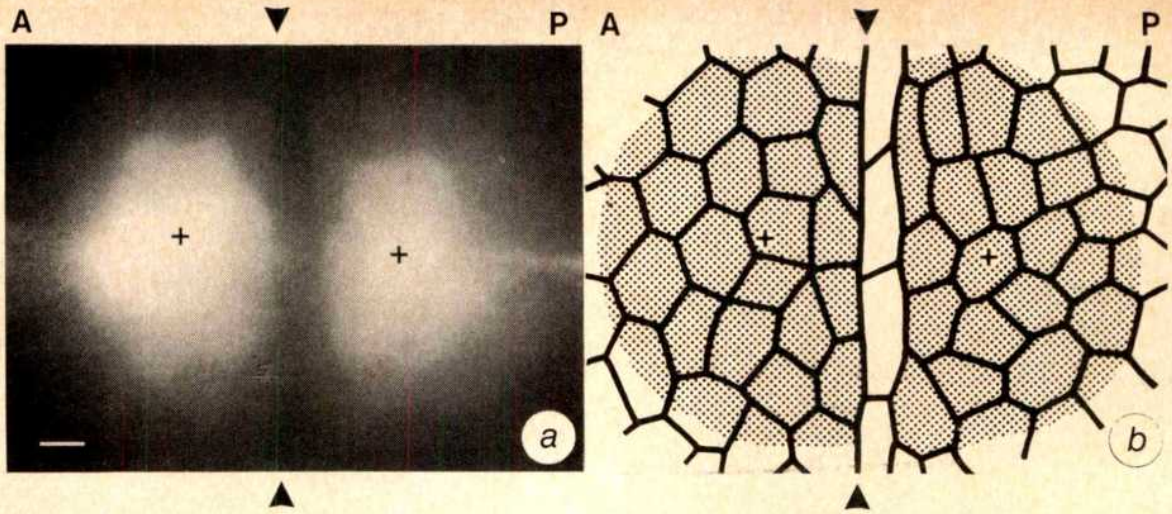


Fig. 3 The cell-to-cell spread of LRB injected into a cell on each side of the border simultaneously outlines a border strip of cells slow to receive the dye. The reduced permeability of these cells may explain how molecular interactions between compartments are limited. Arrowheads mark the position of the segment border. A, anterior segment; P, posterior segment. Scale bar, 10 μm. LRB was injected into the two cells (+) with 200-ms current pulses of 5 nA, 1 pulse per s. In *a*, the extent of spread of LRB was photographed between 20 and 30 s after injection (exposure time 10 s). In this preparation the border strip of cells only partially restricted the segment-to-segment spread of dye. While dye spread extensively among the cells of each segment, it was retarded sufficiently in the border region to outline the border cells. *b*, Margins of the cells seen in *a* drawn from their combined fluorescence and phase-contrast images, showing the extent of the dye spread (stippled areas) from the two injection sites. As in Fig. 2, the border strip (not stippled) appears to consist of a single row of cells.

compartment (similar to a mechanism that may exist at compartment boundaries in the *Drosophila* wing disc[2]). The ability of the border cell junctions to block selectively the passage of organic tracers in certain conditions *in vitro*[1,2] suggests that these cells may modulate the size range of molecules able to pass from segment to segment at different times in development.

Other distinct behaviours of the border cells include their rapid migration and proliferation during wound healing[12,13]. Their reduced junctional communication and characteristic behaviour may allow these cells to remain neutral in their gradient properties[12] and hence serve to establish and maintain discontinuity between the segmental compartments.

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Expression of a V_HC_κ chimaeric protein in mouse myeloma cells

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The heavy (H) and light (L) chains of antibodies consist of variable (V) and constant (C) regions. The V regions of the heavy and light chains form the antibody combining site[1,2]. To determine whether a V region could be functional when joined to a polypeptide other than its own C region, we constructed a chimaeric gene encoding the V region of a mouse heavy chain and the C region of a mouse κ light chain (V_HC_κ). The heavy-chain gene is derived from an A/J mouse hybridoma cell line 36-65 whose antibody product (γ1, κ) is specific for the hapten azophenylarsonate[3]. We report here that, when introduced into a mouse myeloma cell line, the chimaeric gene is expressed and a protein of the expected molecular weight is secreted into the medium. As light chains tend to dimerize[4,5] we expected that the V_HC_κ protein might associate with light chain from the cell line 36-65 to form an antibody-binding molecule. Affinity binding experiments and K_a determination indicate that this is the case. Dimers of this type offer a novel and interesting alternative to existing antibody-binding molecules.

Figure 1 shows the scheme used to construct two plasmids, ΔHpV and ΔHpVE, bearing the V_HC_κ chimaeric gene and the selectable marker Ecogpt[6,7]. Both ΔHpV and ΔHpVE contain the rearranged 36-65 V_H gene with its 5′ flanking sequence[8], and DNA encoding the C region of the κ light chain of the S107 BALB/c mouse myeloma cell line[9]. A chimaeric intron separates the V_H and C_κ genes; this intron includes the light chain transcriptional enhancer sequence (E_κ)[10,11] (V.T.O. and S.L.M., unpublished results) in ΔHpV, and both E_κ and E_H, the heavy chain transcriptional enhancer sequence[12–14], in ΔHpVE (Fig. 1).

ΔHpV and ΔHpVE were separately transfected into J558L BALB/c mouse myeloma cells by protoplast fusion[15] as described elsewhere[16]. (J558L is a variant of the J558 cell line[17] that synthesizes and secretes only a λ light chain but no heavy chain.) Transformants containing the Ecogpt gene were selected in medium containing mycophenolic acid[6,7]. Ten transformants from each of the two transfection experiments were analysed for production of V_HC_κ by immunoprecipitation with rabbit antiserum specific for the C region of mouse κ chain, and separately with rabbit anti-idiotype (Id) antiserum specific for

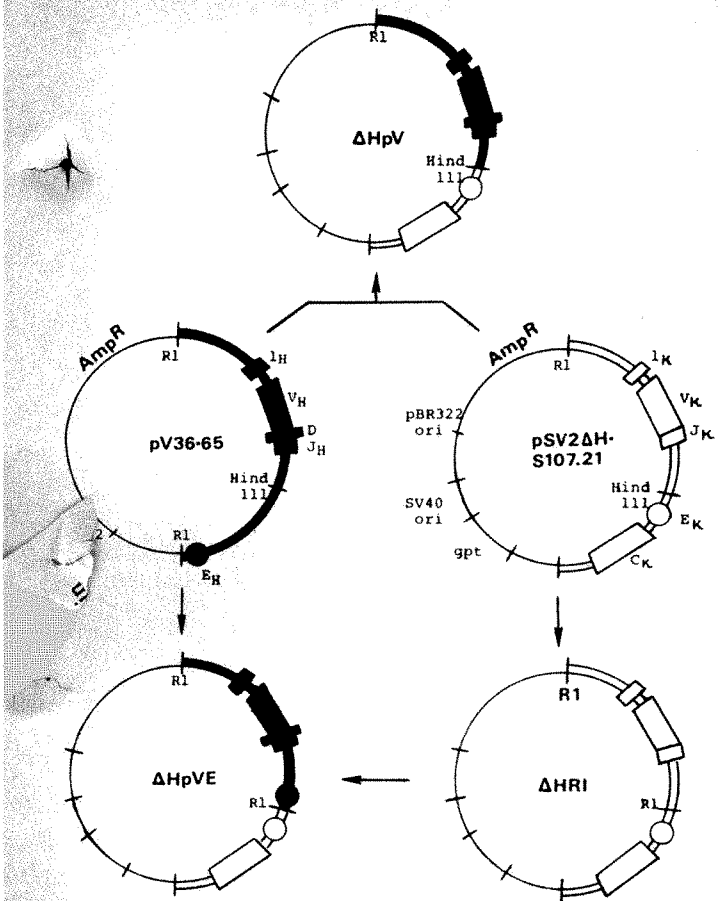


Fig. 1 Construction of V_HC_κ-containing plasmids. pV36-65 contains the 363-base pair (bp) rearranged V_H gene derived from the hybridoma cell line 36-65. This gene is flanked by 2.4 kilobase pairs (kbp) of upstream sequence and 2.1 kbp of downstream sequence terminating in the J_H–C_μ intron[8]. pSV2ΔHS107.21 was derived from pSV2-S107 (ref. 16) by elimination of one *Hin*dIII site. pSV2ΔHS107.21 contains the *Escherichia coli* xanthine–guanine phosphoribosyl transferase gene (*gpt*), and the entire rearranged genomic κ light-chain gene of the S107 myeloma cell line[9] including the leader (1_κ), V_κ and C_κ exons as well as flanking 5′ and 3′ sequences. The *gpt* gene confers on mammalian cells resistance to mycophenolic acid when xanthine is present in the medium[6,7]. The simian virus 40 (SV40) origin of DNA replication (ori) and early promoter are located 5′ to the *gpt* sequence. The S107 κ gene is oriented in the opposite direction to *gpt*. ΔHR1 was constructed from pSV2ΔHS107.21 by converting the *Hin*dIII site into an *Eco*RI site. To generate ΔHpV the *Eco*RI–*Hin*dIII fragment containing the rearranged V_H gene from pV36-65 was substituted for the *Eco*RI–*Hin*dIII fragment containing the rearranged V_κ gene in pSV2ΔHS107.21. To generate ΔHpVE the *Eco*RI–*Eco*RI fragment containing the rearranged V_H gene from pV36-65 was substituted for the *Eco*RI–*Eco*RI fragment containing the rearranged V_κ gene in ΔHR1. The heavy-chain exons and introns are represented by solid boxes and lines; the κ chain exons and introns are represented by open boxes and lines; the heavy-chain enhancer sequence (E_H)[12–14], and the κ chain enhancer sequence (E_κ)[10,11] (V.T.O. and S.L.M., unpublished results) are represented by a solid and an open circle, respectively. Plasmids are not drawn to scale.

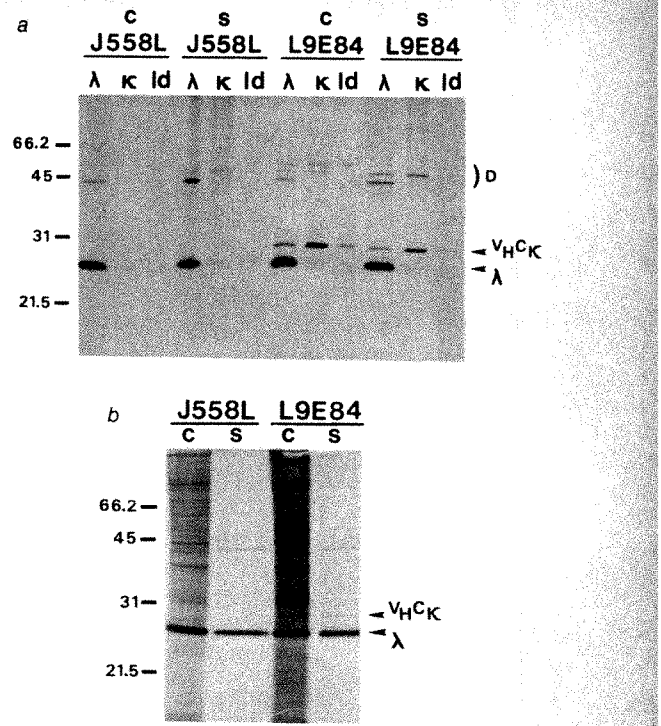


Fig. 2 Autoradiographs of SDS-polyacrylamide gels of cytoplasmic (c) and secreted (s) proteins. Cells were biosynthetically labelled with ^{35}S-methionine in methionine-free Dulbecco's modified Eagle's medium (Irvine Scientific, Santa Ana, California) for 1 h for cytoplasmic protein assay and 3 h for secreted protein assay. Cell extracts were in 0.5% Nonidet P-40 (NP40) containing 0.06 M iodoacetamide. Electrophoresis was in 12.5% SDS-polyacrylamide gels in non-reducing conditions. The positions of the molecular weight markers (Biorad) (in kilodaltons) are indicated; the positions of V_HC_κ and λ light chain are indicated by arrows. ***a***, Cytoplasmic and secreted proteins from J558L and from L9E84 cells were immunoprecipitated with rabbit anti-mouse antisera and *Staphyloccocus aureus*[26] (Staph A) and electrophoresed as described[27]. The antisera used are indicated above each lane. Rabbit anti-mouse κ was obtained from M. Potter under the auspices of NCI contract NO1-CB-25584. Rabbit anti-mouse α + λ (designated λ) was prepared in the laboratory of S.L.M. by L. A. Wims. Rabbit anti-Id antiserum was prepared by L. J. Wysocki. Dimers (D) are indicated. ***b***, Total cytoplasmic and secreted proteins from J558L and from L9E84 cells. Densitometer tracings of L9E84 lanes were done using a Quick Scan (Helena Laboratories). For quantitation, peaks from Xerox copies of the scans were cut out and weighed.

the V_H protein sequence[3,18]. Whereas 6 out of 10 ΔHpVE transformants produced a protein reactive with the anti-κ and anti-Id antisera, only one out of 10 ΔHpV transformants produced such a protein. The relative amounts of the chimaeric protein produced by ΔHpV and ΔHpVE transformants were not significantly different (not shown). The greater number of positive transformants obtained with ΔHpVE might reflect the presence of the heavy-chain enhancer in ΔHpVE.

The ΔHpV positive transformant (which was the first to be recovered) was subcloned in soft agarose[19,20], and one clone, L9E84, was selected for further studies. Figure 2*a* shows an immunoprecipitation analysis of biosynthetically labelled cells. A protein of the expected molecular weight (29,500) reactive with anti-κ and with anti-Id antisera is present in L9E84 but not in J558L cell extracts. Furthermore, this protein is secreted into the medium (Fig. 2*a*). Immunoprecipitation of V_HC_κ from L9E84 culture supernatant by anti-κ antiserum removed the species reactive with anti-Id antiserum (not shown). The V_HC_κ chimaeric protein can be differentiated from the J558L λ light chain by its lower mobility in SDS-polyacrylamide gels in non-reducing conditions (Fig. 2). The upper bands in Fig. 2*a* are probably covalent dimers (D); from the relative sizes of these putative dimers it seems that V_HC_κ can dimerize both with itself and with the J558L λ light chain.

The V_HC_κ chimaera comprises about 1% of the biosynthetically labelled L9E84 cell protein and is present in one-tenth the amount of J558L λ light chain, as determined by densitometer tracing of the autoradiogram shown in Fig. 2*b*.

Ascites from BALB/c mice injected intraperitoneally with L9E84 cells contains the chimaeric protein, as determined by radioimmunoassay. This assay measured the ability of the ascites fluid to compete with the ^{125}I-labelled idiotype-bearing molecule, 36-65 Fab, for the binding of rabbit anti-Id antiserum[3].

mRNA encoding the V_HC_κ protein in L9E84 cells was detected by Northern analysis (Fig. 3). An RNA species of the expected size, ~1.3 kilobases (kb), that hybridizes with an azophenylarsonate (Ars) idiotype probe (specific for the V_H sequence) and with a κ probe (specific for the C_κ sequence) is present in L9E84 but not in J558L cells. It is noteworthy that the chimaeric intron between V_H and C_κ is probably correctly spliced using the donor splice site from the heavy-chain gene and the acceptor splice site from the κ light-chain gene. Correct splicing of a chimaeric intron was observed previously[21].

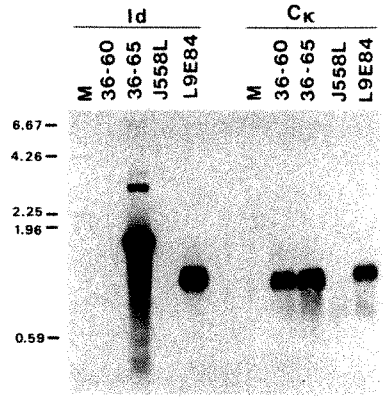


Fig. 3 Northern analysis of L9E84 and control cell RNA. Total cell RNA was prepared from culture cells as described elsewhere[28]. The RNA (6 μg per lane) was electrophoresed in duplicate in a 1.2% agarose gel in 10 mM Na-phosphate and transferred to nitrocellulose[29]. The nitrocellulose filter was cut in two and the RNA hybridized to nick-translated ^{32}P-labelled probes. One half was hybridized to a 133-bp V_H probe specific for the V_H gene encoding the anti-Ars idiotype-positive heavy chain[8,30], while the other was hybridized to a mouse C_κ region cDNA probe (obtained from A. Bothwell and D. Baltimore). The cell line from which the RNA was derived is indicated above each lane. 36-60 is an A/J-derived hybridoma which produces an antibody specific for Ars but devoid of the Ars cross-reactive idiotype[3], and uses an unrelated V_H gene. A ^{32}P-labelled *Hin*dIII digest of phage λ DNA was used as a molecular weight marker (M), and fragment sizes are indicated in kbp. The two halves of the nitrocellulose filter were reattached for autoradiography.

We wished to determine whether V_HC_κ would associate with the homologous 36-65-derived κ light chain to form an antigen-binding dimer; such a dimer would be analogous to a Fab fragment, except that it would have two C_L domains. It has been shown that V_H and V_L can associate to form a fully active antibody-combining site or Fv fragment[1,2]. Furthermore, X-ray crystallographic evidence suggests that in a light-chain dimer one monomer is oriented as the light chain while the other simulates the heavy chain in an antigen-binding Fab fragment[22]. It was therefore expected that a V_HC_κ–$V_\kappa C_\kappa$ dimer would function as a Fab fragment. For this experiment the source of 36-65κ chain was cell line 36-65L, a variant of the 36-65 cell line that synthesizes and secretes only the κ light chain and no heavy chain (isolated by J.S.). When V_HC_κ and the homologous 36-65κ light chain were associated *in vitro*, the resulting molecule bound to Ars-bovine γ-globulin (Ars-BGG)–Sepharose. SDS-polyacrylamide gel electrophoresis of the affinity-bound protein (Fig. 4*Ab*, MIX) and densitometric analysis showed that the V_HC_κ and the 36-65κ chain were present in a 1:1 molar ratio. Binding to Ars-BGG–Sepharose was significantly reduced by competition with Ars-tyrosine hapten (Fig. 4*Ac*, MIX). λ Chain, [illegible] amounts of ~30% of the κ or V_HC_κ chain, also bound to the Ars-BGG–Sepharose (Fig. 4*Ab*, MIX); this binding is evidently nonspecific because, unlike that of the κ and V_HC_κ chains, it is not competed for by the Ars-tyrosine hapten (Fig. 4*Ac*, MIX). L9E84 cells produce large amounts of this chain (see Figs 2*b*, 4*Aa*), and ~2% of the input chain binds to the Ars-BGG resin.

These data suggest that a V_HC_κ chimaera can associate with light chain *in vitro* to form an antigen-binding molecule. We estimate, from densitometer tracing of gel autoradiograms (not shown), that 20–30% of the V_HC_κ protein had associated with 36-65 light chain to form a functional binding site.

We have recently succeeded in introducing the V_HC_κ gene into 36-65L cells by fusion of 36-65L cells with myeloma cells that had been transfected with the V_HC_κ gene (J.S., unpub-

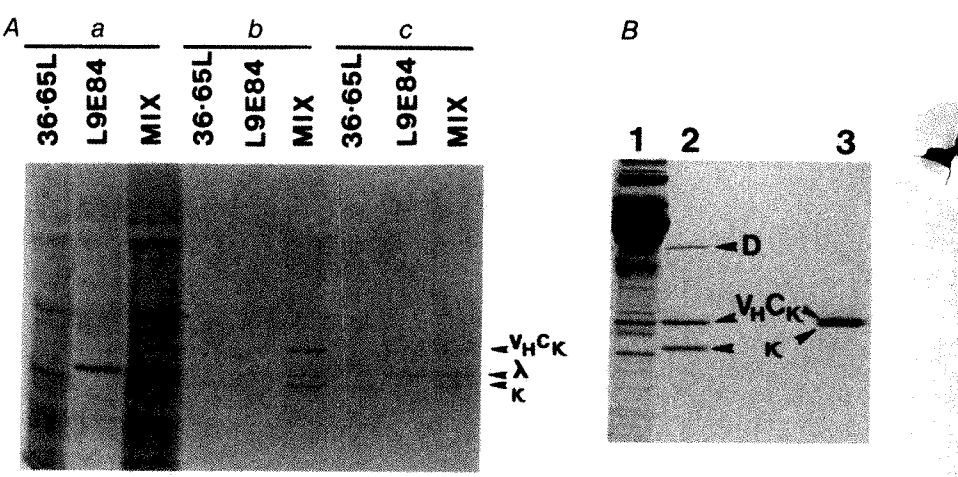


Fig. 4 Association of V_HC_κ with 36-65 κ light chain to form an antigen-binding molecule. *A*: *a*, Dialysed cell extracts and mix preabsorbed with BGG–Sepharose. One-fifth the amount used for binding to Ars-BGG–Sepharose was applied to the gel. *b*, Protein bound to Ars-BGG–Sepharose. *c*, Protein bound to Ars-BGG–Sepharose in the presence of 0.5×10^{-3} M Ars-tyrosine (ABA-tyrosine from Bioresearch, San Rafael, California). The positions of V_HC_κ, λ and 36-65 κ chain are indicated. *B*, SDS-polyacrylamide gel electrophoresis of V_HC_κ–$V_\kappa C_\kappa$ dimers purified from 25D3 ascites. Lane 1, 2 μl of ascites, in non-reducing conditions; lane 2, purified V_HC_κ–$V_\kappa C_\kappa$ dimers in non-reducing conditions; lane 3, purified V_HC_κ–$V_\kappa C_\kappa$ dimers, in reducing conditions. The positions of V_HC_κ, κ and covalent dimers (D) were determined from their ^{35}S-biosynthetically labelled counterparts in cell line 25D3, and are indicated by arrows.

Methods: *A*, L9E84 and 36-65L cells were biosynthetically labelled separately with ^{35}S-methionine and cell extracts prepared as described in Fig. 2 legend. Portions of 36-65L, L9E84 and an equal mixture of the two extracts (MIX) were made up to 0.5 M in propionic acid to dissociate interchain noncovalent interactions. After 30 min at room temperature the individual extracts and the mix were dialysed exhaustively against 5 mM of Na-acetate *p*H 5.5 at 4 °C to allow association into dimers[31,32], and finally against phosphate-buffered saline (PBS) at room temperature. The dialysed extracts and the mix were preabsorbed with BGG-coupled Sepharose for 2.5 h at 4 °C to reduce nonspecific binding, and then portions of each were mixed with 15 μl of Ars-BGG–Sepharose for 10 h at 4 °C in Eppendorf tubes. The resin aliquots were washed five times with 1 ml of PBS, 1 M NaCl, 0.5% NP40, once with 1 ml of ethylene glycol[33,34] in the above solution, and once with 1 ml of PBS. Bound protein was eluted with SDS sample buffer at 100 °C[35]. SDS-polyacrylamide gel electrophoresis in non-reducing conditions, and auto-radiography were as described[27]. Densitometer tracing and quantitation were done as described in Fig. 2 legend. (The ratio of methionine residues in V_HC_κ to those in 36-65 κ is 3:2.) *B*, Ascites was produced in (BALB/c × A/J) F_1 mice by intraperitoneal injection of 10^5 25D3 cells after priming with 0.5 ml of pristane (2,6,10,14-tetramethylpentadecane; Aldrich)[36]. Dimers with antigen-binding activity were purified by affinity chromatography on Ars-BGG–Sepharose. Elution was with 0.2 M NH_4OH. The eluted material was chromatographed on an Ultrogel AcA 44 column (LKB) that had been calibrated with molecular weight markers; the bulk of the material was recovered as a 55,000-molecular weight protein peak. SDS-polacrylamide gel electrophoresis was performed as described above, and proteins were visualized by Coomassie blue staining.

lished). This resulted in *in vivo* association of V_HC_κ with the light chain of 36-65. Formation of functional antibody molecules *in vivo* following transfection of heavy and light chains into myeloma cells has been demonstrated[23]. Ascites from (BALB/c×A/J)F_1 mice that had been injected with the hybrid cell line, 25D3, contains the V_HC_κ–$V_\kappa C_\kappa$ dimer. This dimer was purified by affinity chromatography on Ars-BGG–Sepharose. Gel filtration chromatography indicated a molecular weight of 55,000, as expected. SDS-polyacrylamide gel electrophoresis showed the presence of V_HC_κ, κ and covalent dimers (Fig. 4*B*).

Measurements of the fluorescence quenching of the V_HC_κ–$V_\kappa C_\kappa$ dimer and of 36-65 hybridoma protein by Ars-tyrosine were made as described[24] and used to calculate K_a. The K_a values for the association of V_HC_κ–$V_\kappa C_\kappa$ and that of the 36-65 protein with Ars-tyrosine (assuming one binding site per molecule for the V_HC_κ–$V_\kappa C_\kappa$ dimer and two binding sites per molecule for the 36-65 protein) are essentially the same: $3.8\times10^5\ M^{-1}$ and $4.2\times10^5\ M^{-1}$, respectively. A similar value was determined previously for hybridoma protein 36-65 (ref. 24).

It would be interesting to compare X-ray crystallographic studies of the V_HC_κ–$V_\kappa C_\kappa$ dimer and of the Fab fragment of 36-65 protein. The dimer might be a good candidate for crystallization because of its increased symmetry compared with Fab fragments. (It has been observed that some Fab fragments cannot be induced to crystallize[25].) Such structural studies might provide insights into designing antibody molecules consisting of V_H and V_L attached to other molecules of interest such as enzymes and toxins.

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Schwann cells induce morphological transformation of sensory neurones *in vitro*

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Cell–cell interactions are thought to play a crucial part in determining the developmental fate of vertebrate cells and regulating their subsequent differentiation. In the peripheral nervous system, for example, signals from neuronal axons determine whether or not some Schwann cells wrap their plasma membrane concentrically around the axon to form a myelin sheath[1,2]. Moreover, there is some evidence that the interactions between Schwann cells and neurones are not all one way: for example, Schwann cells are thought to provide signals for neuronal sprouting and regeneration[3–5]. However, there are no clear examples in which Schwann cells have been shown to influence the normal development of neurones. Here I have used purified populations of embryonic sensory neurones and Schwann cells to demonstrate that Schwann cells have a dramatic influence on the development of these neurones. In the presence of Schwann cells, but not other cell types, the sensory neurones undergo a morphological transformation from an immature bipolar form to a mature pseudo-unipolar form. This provides a striking example of the importance of glial cells for neuronal development.

During development in the dorsal root ganglion (DRG) of birds and mammals, sensory neurones are initially bipolar in form, extending one axon to the periphery and the other to the spinal cord. During the second half of development, these neurones undergo a remarkable change in morphology well described by Ramon y Cajal[6]. The cytoplasm of the neuronal cell soma bulges to one side and the two axons approach one another so that the cell becomes bell-shaped. Eventually the cytoplasm between the cell body and the processes elongates to form the initial segment of the axon from which the original processes emanate (see Fig. 1). This change from bipolar to pseudo-unipolar morphology is associated with a rearrangement of the cell body cytoplasm and cytoskeleton[7,8].

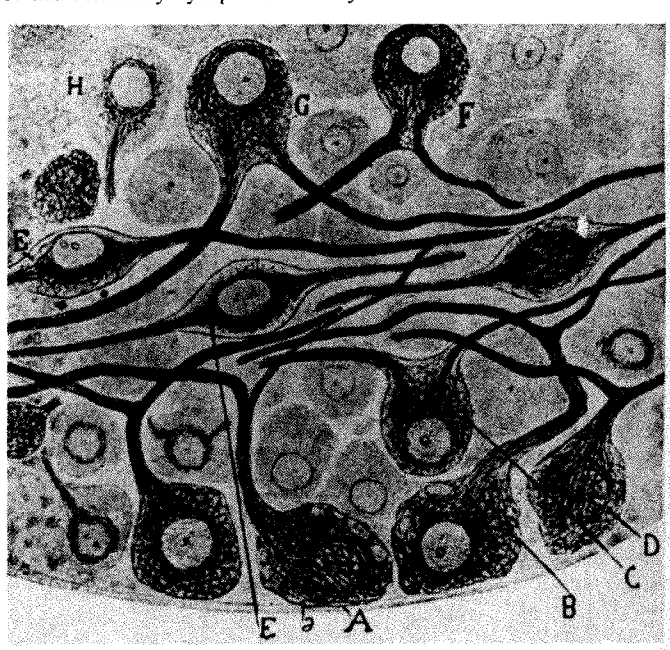


Fig. 1 DRG neurones from 14-day-old chick embryo, stained with silver nitrate. This drawing which shows the transition of bipolar sensory neurones to the pseudo-unipolar form is reproduced from ref. 6.

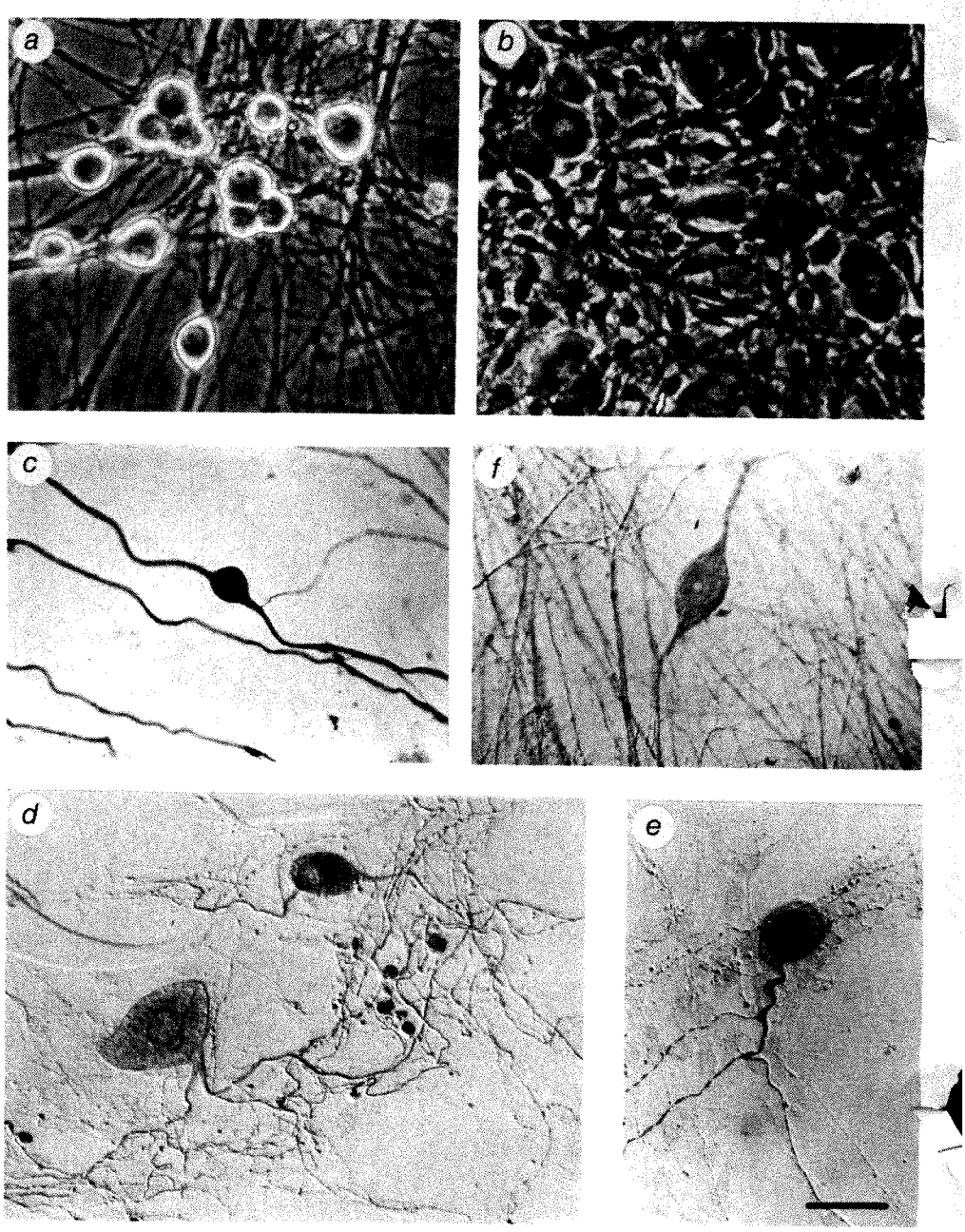


Fig. 2 *a*, *b*, Phase-contrast micrographs of sensory neurones grown for 10 days without the addition of Schwann cells (*a*), or grown for 5 days after the addition of Schwann cells (*b*). *c*, Sensory neurone grown for 15 days on a collagen substrate in the absence of non-neuronal cells. *d*, Sensory neurones 10 days after the addition of Schwann cells. The neurones appear to be undergoing a transition to the pseudo-unipolar form. *e*, Sensory neurone 10 days after the addition of Schwann cells. *f*, Sensory neurone grown on extracellular matrix material laid down by bovine endothelial cells. Scale, 50 μm.

Methods: ***a***, ***b***, DRGs from 9–10-day-old chick embryos were dissociated using 0.1% Dispase (Boehringer Mannheim) in Ca^{2+},Mg^{2+}-free saline and plated onto collagen-coated 35-mm tissue culture dishes (Falcon) or 16-mm glass coverslips in 24-multi-well trays (Linbro). Cells were grown in minimal essential medium containing 10% heat-inactivated horse serum, 5% chick embryo extract, 2 mM glutamine, 50 μg ml^{-1} penicillin, 50 U ml^{-1} streptomycin and 1 μg ml^{-1} nerve growth factor as previously described[9,10]. The cultures were treated with 5×10^{-6}M cytosine arabinoside for 5 days to eliminate non-neuronal cells. At this time, purified Schwann cells were added to some of the neurone-alone cultures. The plating density of the Schwann cells was either 200,000 per 35 mm dish or 50,000 per 19 mm multi-well with coverslip. ***c–f***, Sensory neurones plated at low density and labelled with anti-NF antibody viewed with Nomarski optics. Cultures were fixed with 4% paraformaldehyde followed by extraction with cold methanol. The extracted cells were then incubated with a monoclonal antibody (RT 97)[12] to neurofilament proteins, washed and then incubated with anti-mouse Ig conjugated to peroxidase (Tago). The bound antibody was then visualized by reacting with diaminobenzidine (0.05%) and H_2O_2 (0.01%).

In the present experiments, DRGs were dissected from 9–10-day chick embryos, dissociated to single cells and plated onto collagen-coated glass coverslips or plastic tissue-culture dishes. The cultures were treated for 5 days with cytosine arabinoside (5×10^{-6} M) to kill most of the ganglionic non-neuronal cells[9,10]. After this period purified Schwann cells were added to some of the neuronal cultures. The Schwann cells were obtained from newborn rat sciatic nerves and purified free of fibroblasts by pulsing with cytosine arabinoside (10^{-5} M for 2 days) followed by complement-mediated cell lysis of Thy 1.1-positive fibroblasts according to the method of Brockes *et al.*[11]. After growth for a further 5–15 days, the cultures were photographed, then fixed and labelled with a monoclonal antibody against neurofilament proteins (RT97; anti-NF)[12].

Most of the neurones in cultures of 9–10-day-old embryonic chick DRGs were phase bright and bipolar, and maintained this morphology for periods of up to 1 month if grown in the absence of non-neuronal cells (Fig. 2*a*, *c*). However, when purified Schwann cells were added, the morphology of the neurones changed dramatically within 5 days (see below). When viewed with phase-contrast optics, all the neurones had become flat and phase-dark; moreover, the initial segment of the axons appeared thicker (Fig. 2*b*). Labelling the mixed cultures with anti-NF antibody showed that all the neurones had changed from the bipolar to the pseudo-unipolar form. Figure 2*c–e* shows neurones grown at low density with and without the addition of Schwann cells and labelled with anti-NF. This set of cultures was chosen for illustration because they clearly show the intermediate forms reminiscent of Ramon y Cajal's drawings (Fig. 2*d*).

The time required for this change to occur was related to the number of Schwann cells added: with high densities of Schwann cells the change occurred in less than 1 week, the first 'flattening' being observed after 3 days. With low numbers of Schwann cells, the change occurred after a longer period (up to 15 days); however, when the neurones eventually flattened, the majority did so over a period of a few days. Although the dependence on Schwann cell number and the synchrony of the transition suggested that a secreted factor might be responsible for the effect, attempts to induce the morphological change with super-

natants from Schwann cell cultures were unsuccessful. This suggests that neuronal–Schwann cell contact may be required.

Of the cells tested, only Schwann cells were able to induce the morphological change in DRG neurones. Fibroblasts from either rat or chick skin, chick myotubes, rat cortical astrocytes, or the glial cell lines RN22 and C6, had no effect on neuronal morphology when added to DRG cultures. Changing the composition of the substrate by adding fibronectin and/or laminin to the collagen also had no effect. Similarly, DRG neurones cultured on the extracellular matrix material laid down by bovine endothelial cells[13] remained in the bipolar form after 2 weeks, although this matrix greatly stimulated neurite extension. (Fig. 2*f*). Therefore if extracellular matrix components are involved in the change, these components seem to be specific to the Schwann cells.

The present experiments used early embryonic chick neurones and purified rat sciatic nerve Schwann cells. Fortunately, the mechanism of this signalling between Schwann cells and neurones seems to be conserved between species. Chick rather than rat DRG neurones were studied because of the relative ease of culturing early embryonic chick neurones free of non-neuronal cells; many of the neurones taken from chick embryos older than 10 days (or rat embryos older than 15 days) have already been signalled to become pseudo-unipolar, and when put into culture retain this morphology. Rat Schwann cells were used because of the availability of methods for identifying and purifying these cells; comparable methods for identifying and purifying chick Schwann cells are not available. However, I have found that chick non-neuronal cells enriched for glial cells (using spindle-shape morphology as a criterion) also promote the morphological transformation of chick sensory neurones. Dichter and Fischbach[14] noted the presence of a few 'flat' phase-dark chick DRG neurones, which were probably pseudo-unipolar, in cultures grown in the presence of ganglionic non-neuronal cells that were mostly mesenchymal rather than glial cells. Other workers[15] have described the presence of pseudo-unipolar sensory neurones in unpurified rat DRG cultures containing both rat neurones and Schwann cells. However, because of the heterogeneity of the cell populations used, no specific interaction between two identified cell types could be identified in any of these three cases.

The present report illustrates the power of using purified cells to determine which of the properties of differentiated neurones are intrinsic to these cells and which depend on interaction with other cell types. There is a striking similarity between the transformation seen in these cell mixing experiments *in vitro* and that seen during the normal development of sensory neurones *in vivo*. This, together with the fact that the transformation occurs at a time that correlates with Schwann cell proliferation in the DRG[16], suggests that Schwann cells play a crucial part in mediating this morphological change *in vivo* as well as *in vitro*.

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Epstein–Barr virus transforms precursor B cells even before immunoglobulin gene rearrangements

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The very early stages of the human B-cell differentiation pathway are poorly understood, primarily because of the lack of appropriate permanent cell lines. Epstein–Barr virus (EBV) is a putative human oncogenic virus which transforms human B cells *in vitro* into continuously proliferating cells[1]. It has been believed that EBV transforms mature B cells[2,3] but recently, transformation of immature pre-B-cell lines has been reported[4–6], suggesting that EBV might also transform cells much earlier in the B-cell lineage. We report here the establishment of cell lines transformed by EBV at various stages of the B-cell differentiation pathway. Interestingly, two lines showed the complete absence of immunoglobulin synthesis and the lack of immunoglobulin gene rearrangement despite containing EBV genome and surface markers of B cells. Our results indicate that EBV can infect and tranform cells of the B lymphocyte lineage even before immunoglobulin gene rearrangement.

EBV was obtained from the culture fluid of the B95-8 marmoset cell line[7]. The fetal liver was obtained from an embryo artificially aborted at the 16th week of gestation. Mononuclear cells separated by Ficoll–Conray gradient centrifugation were infected with EBV at a multiplicity of infectivity of 0.1. Infected cells were distributed into microculture plates (no. 3042, Falcon) at a density of 1×10^5 cells per well in RPMI 1640 medium supplemented with 20% heat-inactivated fetal bovine serum, 0.2 mM L-serine, 1 mM pyruvic acid, 2 mM L-glutamine, 100 U ml^{-1} of penicillin, 100 $\mu g\ ml^{-1}$ of streptomycin and 10 mM HEPES and were cultured at 37 °C in an atmosphere of 5% CO_2 in air[8]. After 30–40 days, transformation occurred in 30% of the wells, as determined by the morphological appearance of lymphoblastoid cells, increase of cell numbers and acid production.

The immunoglobulin phenotypes of 24 cell lines thus obtained were examined (Table 1). The various cell lines showed different immunoglobulin production and were classified into four groups. Of the 24 cell lines, 20 were IgM producers which had both cytoplasmic and surface μ and light chains. The amounts of IgM secreted in culture fluid varied over 270–52,500 ng ml^{-1}. The light chains made by all of these cell lines except FLEB5 were κ isotype. This finding may reflect the dominant expression of κ isotype in the fetal liver B cells, and supports the hypothesis that the κ-chain gene is activated before the λ-chain gene in human B-cell ontogeny[9]. Another cell line, FLEB12, synthesized only μ chains in the absence of light chains and therefore appeared to represent a pre-B cell[10,11]. Using the method of double colour staining[12], 60% of the FLEB12 cells possessed μ chains only in the cytoplasm but 10% had μ chains both in the cytoplasm and on the cell surface (data not shown). This result supports the proposal that μ and light chains may not need to be assembled for expression on the cell surface. In the case of line FLEB20, only κ chains were detected. This might be a similar cell line to those mouse cell lines reported by Siden *et al.*[13] which produce light chains only but have rearranged heavy-chain genes[14]. Unfortunately, the FLEB20 was subsequently lost. The remaining two cell lines, FLEB14 and 18, showed complete absence of immunoglobulin synthesis.

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Table 1 Immunoglobulin phenotypes of cell lines

Cell lines	Secreted immunoglobulin (ng ml^{-1})		Surface immunoglobulin (%)						Cytoplasmic immunoglobulin (%)					
	IgM	IgG	μ	γ	δ	α	λ	κ	μ	γ	δ	α	λ	κ
FLEB 1	820	0	0.6	0	0	0	0	0.2	0.8	0	0	0	0	0.7
2	15,000	0	37	0	0	0	0	39	50	0	0	0	0	57
3	550	0	0.8	0	0	0	0	0.4	1.2	0	0	0	0	0.5
4	1,950	0	2	0	0	0	0	1	3	0	0	0	0	0.5
5	12,000	0	14	0	0	0	16	0	25	0	0	0	19	0
6	270	0	0.8	0	0	0	0	0.1	0.4	0	0	0	0	0.1
7	350	0	1	0	0	0	0	0.3	0.9	0	0	0	0	0.8
8	2,460	0	5	0	0	0	0	2	6	0	0	0	0	3
9	890	0	0.1	0	0	0	0	0.6	1.5	0	0	0	0	0.3
10	1,400	0	0.2	0	0	0	0	0.1	0.2	0	0	0	0	0.1
11	750	0	1	0	0	0	0	2	1	0	0	0	0	2
13	1,300	0	1	0	0	0	0	2	1	0	0	0	0	0.8
15	290	0	0.1	0	0	0	0	0.1	0.3	0	0	0	0	0.2
16	48,000	0	52	0	0	0	0	40	38	0	0	0	0	41
17	280	0	0.7	0	0	0	0	0.8	1	0	0	0	0	0.6
19	2,500	0	2	0	0	0	0	2	2	0	0	0	0	0.8
21	440	0	0.4	0	0	0	0	1	0.7	0	0	0	0	2
22	52,500	0	38	0	0	0	0	22	33	0	0	0	0	27
23	680	0	1	0	0	0	0	2	3	0	0	0	0	1
24	540	0	0.6	0	0	0	0	0.2	0.8	0	0	0	0	0.1
12	57	0	11	0	0	0	0	0	66	0	0	0	0	0
20	0	0	0	0	0	0	0	8	0	0	0	0	0	11
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The amounts of IgG and IgM were determined by an inhibition radioimmunoassay described by Platts-Mills and Ishizaka[24]. Briefly, immunoadsorbent column-purified IgG and IgM were labelled with ^{125}I(NEN) by the chloramine-T method. 100 µl of appropriately diluted supernatants and standard human IgG (or IgM) were mixed with 0.1 ml of rabbit anti-human IgG (or anti-human IgM) serum. After 2 h incubation at room temperature, 0.1 ml of ^{125}I-labelled IgG (or IgM) (59 ng ml^{-1}) was added and incubated for another 2 h at room temperature. Then 0.1 ml of goat anti-rabbit IgG serum was added and allowed to stand overnight at 4 °C. The precipitates were spun down and radioactivity was counted using an autogamma-counter. Cytoplasmic and surface immunoglobulins were detected by direct immunofluoresence. The cells were smeared on a coverslip, fixed in acetone at −20 °C and stained for cytoplasmic immunoglobulin with fluorescein isothiocyanate (FITC)-coupled rabbit monospecific antisera, F(ab')$_2$ fragment, against human μ, δ, γ, α, κ and λ chains. Surface immunoglobulin was stained by the method of Kumagai *et al.* after treatment with acid *p*H[25].

Three representative cell lines (FLEB2, 12 and 14) were subcloned in semi-solid agarose. Each subclone conserved the same characteristics as the parental cell lines. To confirm the immunoglobulin phenotypes of three subcloned lines (FLEB2, 12 and 14), the cells were metabolically labelled with ^{14}C-leucine and their cell lysates and culture fluids were immunoprecipitated with purified anti-μ serum. The precipitated polypeptides were analysed by SDS–polyacrylamide gel electrophoresis (PAGE) (Fig. 1). In the lysate (lane 1) and culture fluid (lane 4) of FLEB2 the molecular weights (MWs) of two observed polypeptides were 76,000 and 29,000, corresponding to those of authentic μ and light chains purified from human serum. In the case of FLEB12 (Fig. 1, lanes 2, 5), two μ-related polypeptides were detected in the lysate but no precipitable polypeptides were observed in the culture fluid. The minor band of the lysate was 76,000 MW and identical in size to the standard μ chain, while the major one was ~80,000 MW. This higher molecular weight may result from differences in the length of these polypeptides and/or in the degree of glycosylation. Alternatively, it may not be a μ-gene product, such as the supposed heavy-chain binding protein (BiP) noncovalently bound to heavy chains reported in murine pre-B-cell lines[15]. The anti-μ antibody did not precipitate any molecules at all in the case of FLEB14 (lanes 3 and 6). Thus, FLEB14 was completely lacking in immunoglobulin expression.

To determine whether FLEB14 belonged to the B-cell lineage or not, the lines were tested for various other markers (Table 2). Like the other cell lines tested, FLEB14 possessed Fc μ receptors, C3 receptors and HLA-DR antigens, which are characteristic of B cells, although not unique to them. They lacked any T-cell markers and peroxidase activities. In addition, FLEB14 was positive for EBV-induced nuclear antigens

Table 2 Other various markers of cell lines

	FLEB2	FLEB12	FLEB14
EBNA	+	+	+
Fc μ receptor	8%	24%	27%
Fc γ receptor	0%	0%	0%
C3b receptor	34%	82%	15%
C3d receptor	7%	18%	9%
HLA-DR antigen	+	+	+
SRBC receptor	−	−	−
T-cell antigen			
Leu 1	−	−	−
Leu 2a	−	−	−
Leu 3a	−	−	−
Leu 4	−	−	−
TdT	−	−	−
Peroxidase	−	−	−
CALLA	3%	19%	0%

EBNA was detected according to the method of Reedman and Klein[16]. Rosette formation of the cells was performed by the method of Tachibana *et al.*[26]. Neuraminidase-treated sheep erythrocytes (SRBC) were used for E-rosette formation. Cells with IgG-Fc or IgM-Fc receptors were tested with IgG- or IgM-coated ox erythrocytes. IgM–human complement-coated SRBC and IgM-C5-deficient mouse complement-coated SRBC were used for detection of C3b and C3d receptors. HLA-DR antigen, CALLA(J5)[27] and T-cell differentiation antigens (Leu 1, Leu 2a, Leu 3a, Leu 4) were tested by fluorocytometry using murine monoclonal antibodies (Becton Dickinson). Terminal deoxynucleotidyl transferase (TdT) activity was determined according to the method of Tofen *et al.*[28]. Peroxidase staining was performed by a standard method.

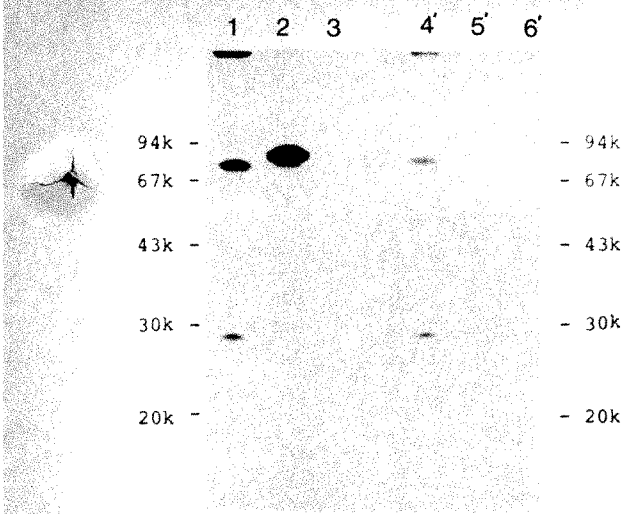


Fig. 1 Lanes 1, 2 and 3 are from cell lysates of FLEB2, 12 and 14. Lanes 4, 5 and 6 are from culture supernatants of FLEB2, 12 and 14. Cells were cultured at 5×10^6 cells per ml with 5 μCi ml^{-1} of ^{14}C-leucine (NEN) for 2–12 h in leucine-free minimal essential medium supplemented with 10% heat-inactivated fetal calf serum. After labelling, the culture media were saved. The cells were washed wice with cold phosphate-buffered saline and were lysed in lysis uffer containing 10 mM sodium phosphate *p*H 7.4, 0.1 M NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1 mg ml^{-1} of bovine serum albumin and 1 mM phenylmethylsulphonyl fluoride. The lysed cells were centrifuged at 100,000*g* for 60 min at 4° C and the supernatants were saved. The spent media and cell lysates were mixed with 10 μl of rabbit anti-human IgM serum. After 12 h incubation at 4° C, 0.1 ml of a 10% suspension of fixed *Staphylococcus aureus* Cowan I was added and incubated for 2 h at room temperature followed by three washings. Bound immunoglobulins were removed and reduced in buffer containing 10 mM Tris-HCl *p*H 6.8, 6.2 M urea, 2.3% SDS and 5% 2-mercaptoethanol for 3 h at 70° C, and *S. aureus* was removed by centrifugation. Reduced samples were separated on a 10–15% gradient polyacrylamide gel according to the method of Laemmli[29]. Following electrophoresis, the gel was fixed and fluorographed.

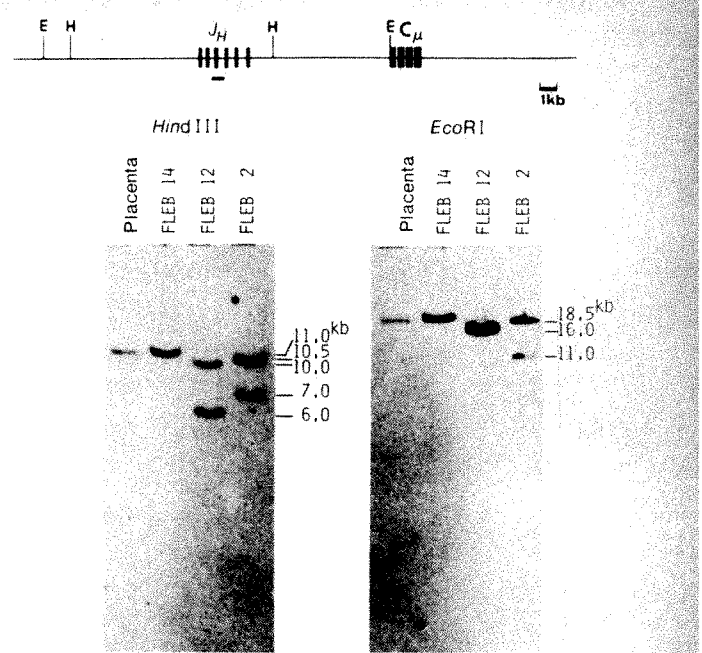


Fig. 2 Immunoglobulin heavy-chain gene analysis of FLEB2, 12 and 14 cell lines with a cloned human J_H gene fragment as a probe. High molecular weight DNA was isolated from each cell line according to the method of S. Gattoni (personal communication)[30]. The human placental DNA was used as a control. The fragments obtained by *Hin*dIII or *Eco*RI digestion of DNA (about 2 μg) were size-fractionated by electrophoresis in a 0.5% agarose gel, transferred to a nitrocellulose filter according to the method of Southern[31], then hybridized with a ^{32}P-labelled cloned J_H gene probe (specific activity 3×10^8 d.p.m. per μg). The probe used was a 0.7-kb *Hha*I–*Hha*I fragment of Ch4A.H.Igμ-24 (ref. 22) which is indicated by a bold bar below the restriction map. The labelling of the probe was performed by nick-translation as previously described[32]. In the restriction map, structural genes are indicated by closed rectangles, and restriction sites of *Hin*dIII and *Eco*RI are shown by H and E, respectively.

(EBNA)[16], thus they must have receptors for EBV[17]. It has been suggested that the presence of EBV receptor could be considered as a B-cell marker[17,18]. The common acute lymphoblastic leukaemia antigen (CALLA), which is limited to expression mainly on pre-B cells[19], was not detected on FLEB14 cells. Therefore, FLEB14 appears to be committed to the B-cell lineage but definitive assignment can be made only if immunoglobulin production can be induced.

As the first event characteristic of B-cell ontogeny is the rearrangement of immunoglobulin genes, it was of interest to know the immunoglobulin gene organization of FLEB14. It has been demonstrated that heavy-chain gene rearrangement precedes that of light-chain genes, and is accomplished by the joining of V_H, D and J_H gene segments[20,21]. The DNA fragments containing J_H before rearrangement, digested by *Hin*dIII or *Eco*RI, are 11.0 or 18.5 kilobases (kb)[22] in length, respectively. The DNAs from cells of three of these lines (FLEB2, 12 and 14) and human placental cells as germ-line control were digested with *Hin*dIII or *Eco*RI (Fig. 2). *Hin*dIII digests of FLEB2 gave J_H positive fragments of 10.5 and 7.0 kb and from FLEB12, fragments of 10.0 and 6.0 kb. None of these corresponded to the fragment of the human placental DNA (11.0 kb). FLEB14, however, showed the fragment of the germ-line size. Similarly in *Eco*RI digestion, J_H-positive fragments of 18.5 and 11.0 kb in FLEB2 and 16.0 kb in FLEB12 were detected, indicating that the immunoglobulin genes had undergone rearrangement in these cell lines. *Eco*RI-digested DNA of FLEB14, however, again produced only one band of 18.5 kb, identical in size to that found in the human placental DNA. These results show that somatic rearrangement of the heavy-chain gene has already occurred in FLEB2 and 12, but that in FLEB14 the heavy-chain gene retains its germ-line configuration. Based on other experiments, the light-chain genes also conserve the embryonic configuration in FLEB14 (M.O., unpublished results).

These cell lines should therefore provide a useful model for studying the regulation of B-cell differentiation. Of particular interest is one cell line, FLEB14, which makes neither heavy nor light chain and has no immunoglobulin gene rearrangement despite carrying B-cell markers. This line may be the first example of the category of 'ur-B cell', proposed recently by Wabl *et al.*[23]. If immunoglobulin gene rearrangement in FLEB14 can be successfully induced, it will provide a system in which to study the earliest molecular aspects of immunoglobulin gene activation. In preliminary experiments, we have obtained some evidence suggesting heavy-chain gene reorganization in FLEB14 during successive cultivation (M.O., to be published).

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Relaxin affects the central control of oxytocin release

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In several species the myometrium is quiescent shortly before parturition[1]. At this time high titres of relaxin are present in the plasma[2] and there is evidence[3] that the hormone has a direct inhibitory action on the uterine muscle. Relaxin could also contribute to uterine quiescence by inhibiting oxytocin release. To determine whether relaxin has a central action on the release of oxytocin, we have studied the effect of intravenous injections of porcine relaxin on milk ejection in the anaesthetized lactating rat. We report that reflex milk ejection was suppressed by relaxin in a dose-dependent manner, the onset of inhibition being rapid and lasting from 10 to 60 min. After the period of inhibition the normal temporal pattern of reflex milk ejection was resumed. Mammary sensitivity to exogenous or endogenous oxytocin was reduced by relaxin but not sufficiently to explain the effects observed. Furthermore, relaxin (1 μg per rat) injected into the cerebral ventricles profoundly disturbed the pattern of reflex milk ejection without affecting the response of the mammary gland to oxytocin. These results suggest a novel role for relaxin within the central nervous system. The site in the brain at which the effects of relaxin are exerted remains unknown.

Rats were taken from the departmental colony where they had been housed in an environment of controlled light and temperature, with water and food available *ad libitum*. The day of delivery was denoted as day 1, and the animals were used between days 8 and 12 of lactation. The protocol for these experiments has been described in detail elsewhere[4]. Briefly, the young rats were separated overnight from their mother to promote mammary engorgement. The following morning the lactating rats were anaesthetized with ethyl carbamate (1.25 g per kg intraperitoneal, i.p.; urethane, BDH) and 2.5 mg intramuscular (i.m.) xylazine (Rompun; Bayer). A saphenous vein and two caudal mammary glands were cannulated and the animal was left for 3 h to stabilize after surgery. Ten hungry young rats were then allowed to suck at the nipples—this stimulus caused the onset of reflex milk ejection characterized by an abrupt and transient rise in intramammary pressure[5], together with the behavioural response of the sucking young. The latency to the first milk ejection was 12 min (mode) with a range of 5–48 min and thereafter occurred every 3–7 min. After at least eight milk ejections had been observed as a control, purified porcine relaxin, prepared by the method of Walsh and Niall[6], was given in 0.2 ml isotonic saline by intravenous (i.v.) injection. Three doses of relaxin were tested (2.5, 5 and 10 μg). Milk ejection was considered to be inhibited if intervals between successive milk ejections were significantly longer than intervals in the pretreatment period (see Fig. 1 for statistical analysis).

Injection of relaxin at all three doses cause a clear suppression of reflex milk ejection (Fig. 2); this effect was dose-dependent

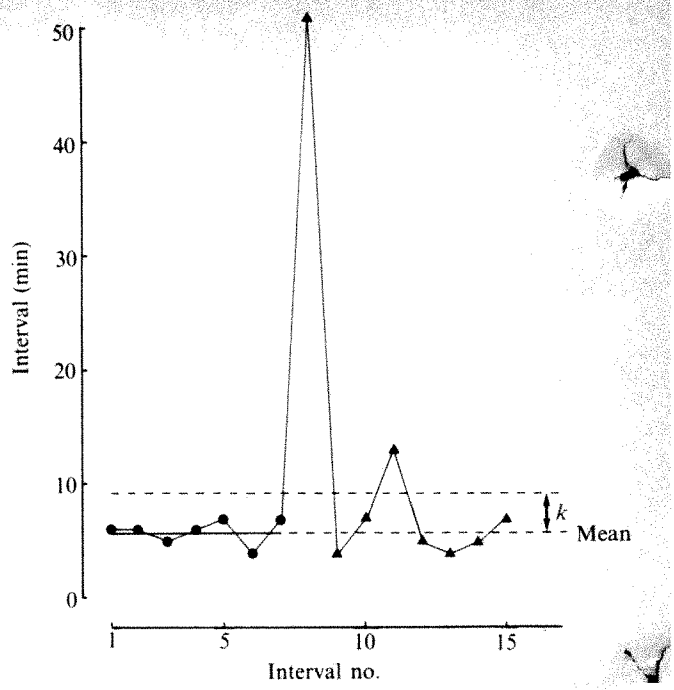


Fig. 1 Analysis used to assess the inhibition of reflex milk ejection by relaxin. This approach was first described by Clarke *et al.*[4]. Intervals between successive milk ejections are plotted sequentially, before (●) and after (▲) injection of relaxin. The lower horizontal line is the mean interval between milk ejections before treatment. The upper dashed line represents a 99% probability estimate ($P = 0.01$) based on the equation $k = \text{s.d.} \times t\sqrt{[(n+1)/n]}$* where s.d. = standard deviation of the milk ejection interval before treatment, n = number of control intervals; t = 't'-value for $n-1$ degrees of freedom at $P = 0.01$ and * = Bessel's correction for small size of preinjection sample. Milk ejection was judged to have been significantly inhibited if any milk ejection interval, within 30 min of relaxin treatment, exceeded the calculated level above.

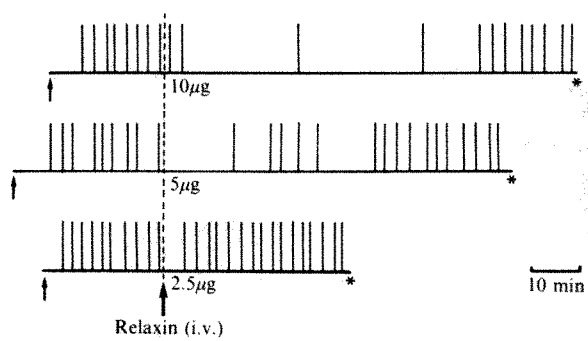


Fig. 2 The effects of intravenous injection of porcine relaxin on the pattern of reflex milk ejection in three suckled, urethane-anaesthetized rats. Each vertical bar corresponds to an intramammary pressure cycle characteristic of milk ejection (↑, onset of sucking stimulus of 10 young rats; *, termination of experiment). The three traces have been aligned with respect to the time of administration of relaxin. Reflex milk ejection was suppressed in a dose-dependent manner (see Table 1).

(Table 1) and lasted from 10 to over 60 min from the time of injection. During periods of inhibition the sensitivity of the mammary gland to oxytocin was tested by i.v. injections of 0.5 mU oxytocin. On all occasions the gland responded to the oxytocin but sometimes after application of 5 or 10 μg relaxin, the amplitude of the pressure response was reduced by up to 50% (Fig. 3), although it was never abolished. After the period of inhibition, reflex milk ejections recommenced with a frequency similar to pretreatment rates. A second dose of relaxin (5 μg) was administered at least 2 h after the initial treatment in four animals; inhibition of the reflex still occurred but to a lesser degree. This might reflect a tachyphylactic response similar to that reported for the myometrium *in vitro*[7].

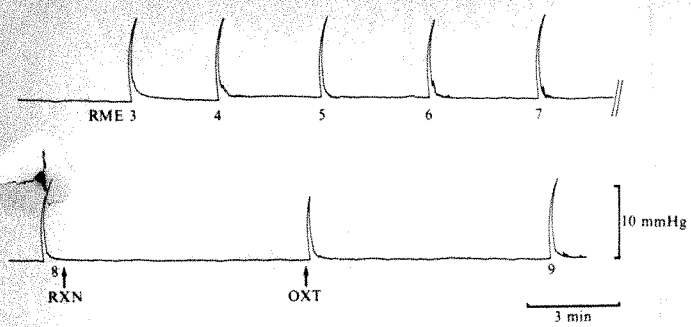


Fig. 3 The effect of relaxin on reflex milk ejection (RME) in an anaesthetized rat. The two traces of intramammary pressure responses are continuous. After the eighth RME, 10 μg relaxin (↑RXN) was injected i.v. Reflex milk ejection was suppressed for 16 min but during this time the mammary gland was still responsive to 0.5 mU exogenous oxytocin (↑OXT), suggesting that treatment with relaxin affected the central release of oxytocin.

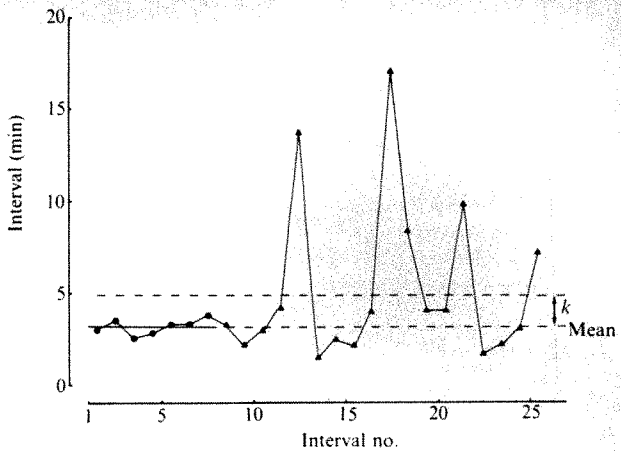


Fig. 4 The effect of intracerebroventricular relaxin on reflex milk ejection. Intervals between successive milk ejections are plotted sequentially before (●) and after (▲) injection of 1 μg relaxin. The lower dashed line is the mean interval between milk ejections before treatment and the upper dashed line represents 99% probability estimation of significant inhibition of the reflex. Reflex milk ejection was clearly suppressed after central administration of relaxin.

We tested the effect on reflex milk ejection of relaxin injected directly into the ventricular system of the brain in six animals. Rats which had been anaesthetized and prepared as described above were placed in a stereotaxic frame and a microsyringe (Hamilton 701N) positioned with its tip in one of the lateral cerebral ventricles. The tip was confirmed to be in the ventricular system by dye injection at the end of the experiment. Reflex milk ejection was initiated by the sucking young and after eight milk ejections, 1 μg relaxin in 1 μl isotonic saline was injected into the ventricle. Relaxin caused a profound disruption in the pattern of milk ejection (Fig. 4) but did not affect the response of the mammary gland to exogenous oxytocin in all six rats. Control injections of 1 μl isotonic saline ($n = 6$) did not affect the temporal pattern of reflex milk ejection. These data imply that the major component of the inhibitory effect of relaxin on milk ejected is exerted within the brain.

Control injections of 0.2 ml isotonic saline (two rats) and an extract of pig ovary (5 μg) shown to possess no relaxin immunoreactivity or bioactivity (uterine strip assay) did not alter the interval between reflex milk ejections in two and three rats respectively. Evidence suggests that electroencephalogram (EEG) synchrony may be a prerequisite for reflex milk ejection in the rat[8,9]. Therefore, we recorded the EEG activity in two anaesthetized lactating rats before and after the i.v. administration of relaxin (5 μg); in neither animal did relaxin alter the EEG activity, suggesting that the hormone did not change the animal's level of arousal. It is also unlikely that the effect of relaxin could be attributed to an action on blood pressure because previous experiments[10] demonstrated that relaxin in doses of up to 20 μg had no effect on arterial pressure.

A possible explanation of the results is that sensitivity of the mammary glands to oxytocin was diminished by relaxin. Therefore, we examined the effect of exogenous oxytocin on intramammary pressure in lactating rats before and after treatment with relaxin. Each injection of hormone (0.5–1 mU) produced an intramammary pressure cycle with a sharp rise and slow decline. These responses mimic the natural pressure changes seen at reflex milk ejection[5], and are relatively constant for a particular dose of hormone[5]. At lower doses the responses are variable; for this reason we used a standard dose of 0.5 mU of synthetic oxytocin (Syntocinon; Sandoz). Rats were anaesthetized and cannulated as described above. After 3 h, 0.5 mU oxytocin was injected i.v. and thereafter at 3-min intervals. A stable baseline of at least seven consistent responses was obtained as control in each animal before relaxin was given; the results are shown in Table 2. Only at doses of relaxin of 5 μg and 10 μg was partial inhibition recorded (Table 2) yet 2.5 μg relaxin caused a suppression of reflex milk ejection in 7 out of 10 rats (Table 1), and in all of six rats 1 μg of the hormone was effective when administered intracerebrally. Furthermore,

Table 1 Effects of porcine relaxin (i.v.) on reflex milk ejection in anaesthetized lactating rats

	Latency (min) to 1st RME [Mode (range)]	RME interval (min) (mean ± s.e.m.) Before relaxin	After relaxin	No. of rats inhibited	Duration (min) of inhibition [Mode (range)]	Milk ejection ratio (mean ± s.e.m.)
2.5 μg Relaxin ($n = 10$)	12 (5–34)	4.5 ± 0.4	5.2 ± 0.3	7/10	10 (4–16)	3.5 ± 0.7
5 μg Relaxin ($n = 10$)	10 (7–48)	4.7 ± 0.8	6.8 ± 0.7	10/10	14 (6–28)	5.9 ± 1.6
10 μg Relaxin ($n = 8$)	12 (6–31)	4.7 ± 0.6	9.9 ± 0.7	8/8	27 (23–>60)	11.0 ± 2.3

The statistical analysis used to assess inhibition is illustrated in Fig. 1. RME, reflex milk ejection. The milk ejection ratio is the duration of the longest interval which occurred within 30 min of relaxin treatment divided by the mean interval before treatment.

Table 2 Effects of porcine relaxin (i.v.) on oxytocin-evoked milk ejection

	No. of rats affected	% Reduction in amplitude of IMP [Mean (range)]	Latency (min) to onset [Mode (range)]	Duration of effect (min) [Mode (range)]
2.5 μg Relaxin ($n = 10$)	0/10	—	—	—
5 μg Relaxin ($n = 10$)	2/10	6.2 (4–90)	3 (3–6)	6 (6–12)
10 μg Relaxin ($n = 12$)	6/12	18.6 (4–92)	3 (3–12)	9 (6–18)

A steady baseline of at least seven consistent responses to 0.5 mU oxytocin given at 3-min intervals was obtained as a control before i.v. injection of relaxin. The reduction in amplitude of the intramammary pressure (IMP) response to exogenous oxytocin after relaxin treatment is expressed as a percentage of the control amplitude.

relaxin at doses of 5 μg and 10 μg only affected the mammary response to exogenous oxytocin for a maximum of 18 min (Table 2), considerably shorter than the period during which reflex milk ejection was suppressed (Table 1). These results indicate that although relaxin also has an effect on the myoepithelium, the block of reflex milk ejection by all three doses of relaxin cannot be explained solely by the peripheral action of the hormone.

Our findings suggest that the inhibitory effect of relaxin on milk yield as reported by Knox and Griffith[11] in rats, and Cowie *et al.*[12] in goats, had a central component. The results also suggest that relaxin reduces *pre partum* myometrial activity by a double mechanism, that is, by a direct effect on the smooth muscle of the uterus and by a central action to prevent oxytocin release.

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Identity of the proliferating cell nuclear antigen and cyclin

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Studies of growth regulation and cellular transformation will be assisted by the identification of proteins that are preferentially synthesized in dividing cells. The 'proliferating cell nuclear antigen' (PCNA), distinguished by its apparent association with cell division, is defined by reaction with an antibody found in the autoimmune disease systemic lupus erythematosus (SLE)[1,2]. This antibody reacts with proliferating cells including tumour cells but gives weak or undetectable immunofluorescence with resting cells of normal tissues[2–6]. Peripheral blood lymphocytes are devoid of PCNA until activated by mitogen *in vitro*. In synchronized cultures its level and distribution fluctuate through the cell cycle, with a striking accumulation in the nucleolus late in the G_1 phase and early in the S phase[3]. Many of these properties are shared by 'cyclin'. This nuclear protein, identified by its position in a two-dimensional separation of cell proteins, is also transformation-sensitive and is preferentially synthesized in the S phase[7–9]. We establish here that PCNA and cyclin are identical, and show that PCNA is an acidic nuclear protein of apparent molecular weight 35,000.

In a recent survey[10], we found anti-PCNA antibodies in about 3% of SLE sera. Immunoglobulins prepared from nine sera of this specificity all precipitated a protein of molecular weight 35,000 from ^{35}S-methionine-labelled HeLa cell extracts (Fig. 1*a*), and we conclude that this protein is the antigen. No evidence was found for an associated RNA species, in contrast to the ribonucleoprotein antigens recognized by certain other antibodies in SLE, but weak labelling of PCNA with ^{32}P-phosphate suggested that it is at least partly phosphorylated (R.M.B.,

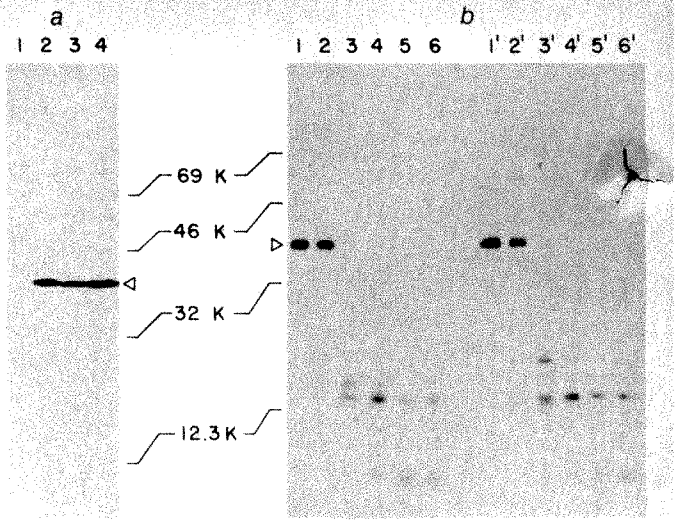


Fig. 1 Immunoprecipitation and peptide analysis of PCNA. *a*, Exponentially growing HeLa cell monolayers were labelled with ^{35}S-methionine for 5 h. Extracts were treated with control human IgG (lane 1) or with anti-PCNA IgG from each of three patients (lanes 2–4). Immune complexes were isolated using *S. aureus* as precipitant and were examined by electrophoresis in a 15% SDS-polyacrylamide gel as described elsewhere (ref. 16 and R.M.B., C. C. Bunn, G. R. V. Hughes, A. M. Francoeur and M.B.M., in preparation). *b*, Protein was extracted from gel bands containing immunoprecipitated PCNA (such as those in *a*) or from the two-dimensional gel spot identified as cyclin (as in Fig. 2*b*), by boiling in a solution containing 0.5% SDS, 10 mM 2-mercaptoethanol and 10 mM Tris-HCl *p*H 7.4. Aliquots of equal radioactivity were digested with protease from *S. aureus* strain V8 and examined in a 20% SDS-polyacrylamide gel. Lanes 1–6, immunoprecipitated PCNA incubated for 15 min at 37 °C with 0, 0.1, 1, 10, 100 and 1,000 ng of V8 protease. Lanes 1′–6′: two-dimensional gel-purified PCNA/cyclin (major spot) treated in the same way. Arrowheads indicate the PCNA band, and the positions of molecular weight markers are shown.

C. C. Bunn, G. R. V. Hughes, A. M. Francoeur and M.B.M., in preparation).

Examination of immunoprecipitates containing PCNA by two-dimensional gel electrophoresis (Fig. 2*a*) revealed a major spot with an apparent isoelectric point of 4.9 and MW ~35,000, together with a more acidic minor satellite spot. Removal of PCNA from the HeLa cell extract using anti-PCNA antibody led to the disappearance of both spots from the two-dimensional gel pattern (compare Fig. 2*b* and *c*), indicating that the spots contain PCNA only. Comparison of the HeLa cell protein pattern (Fig. 2*b*) with published gel patterns showed that PCNA resembles, in its apparent molecular weight and isoelectric point, the spot numbered IEF 49 and named 'cyclin' by Bravo and co-workers[7,8]. The identity of cyclin with PCNA was confirmed by an exchange of samples between our laboratory and that of J. Celis (data not shown), and by construction of one-dimensional peptide maps[10]. The peptides released from immunoprecipitated PCNA by partial proteolysis with *Staphylococcus aureus* V8 protease (Fig. 1*b*) closely resembled those from the two-dimensional gel spot (Fig. 1*b*′) and were similar in size and distribution to those published for cyclin[8,9]. A proteolysis pattern indistinguishable from that shown in Fig. 1*b*′ was also obtained from the acidic satellite spot eluted from a two-dimensional gel (not shown).

PCNA is a prominent component of many tumour cell lines, including HeLa. To examine the biological properties of the protein, we used the REF52 line of rat embryo cells and its virus-transformed derivatives[11]. Cultures of normal REF52 cells have a doubling time of 24 h and reach quiescence at a relatively low cell density, while Simian virus 40 (SV40)-transformed REF52 cells double in 18–19 h and reach a six- to eight-fold higher saturation density. PCNA was readily detected in a long-term labelled culture of proliferating REF52 cells (Fig. 3*a*),

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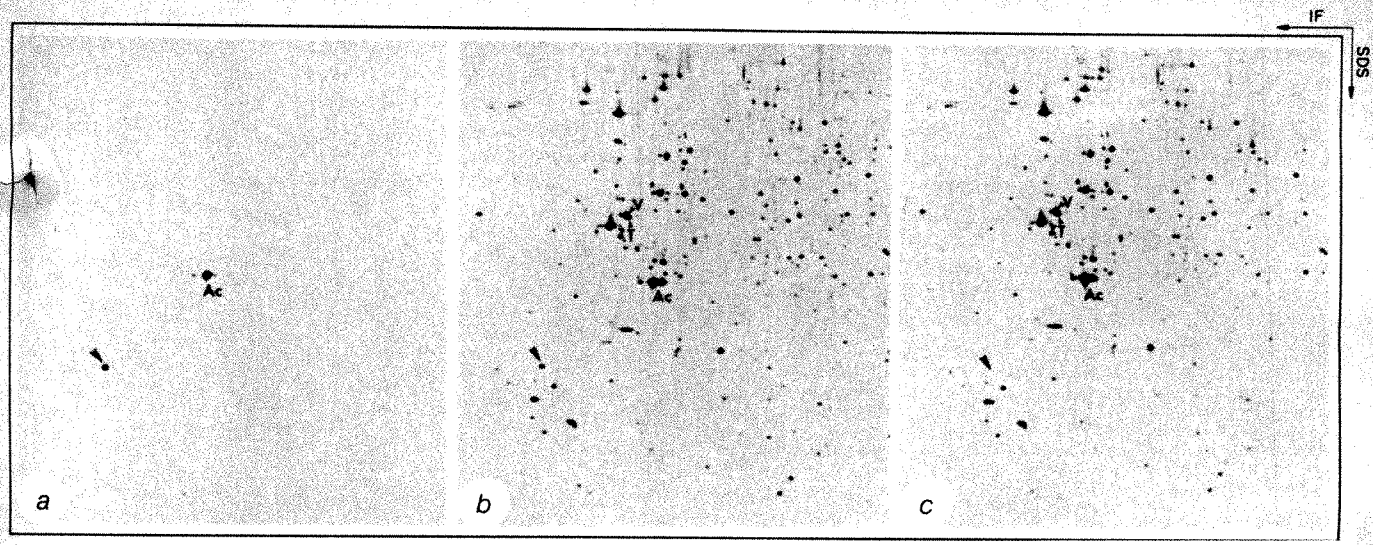
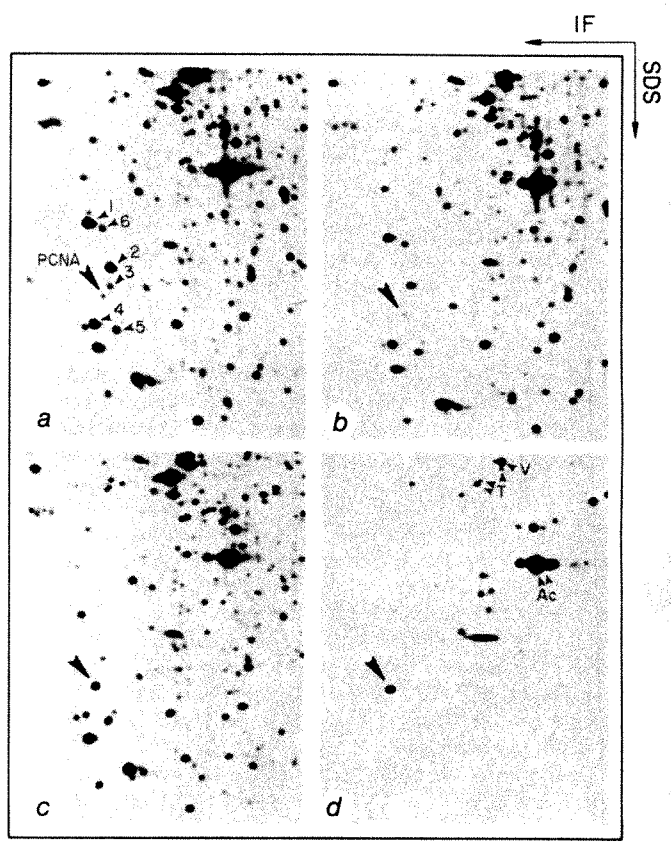


Fig. 2 Identification of PCNA in a two-dimensional gel. PCNA was precipitated from a HeLa cell extract as described in Fig. 1 legend. Proteins of the immunoprecipitate (*a*), the original extract (*b*) and the extract cleared of PCNA by anti-PCNA antibody (*c*), were resolved in two-dimensional gels using *p*H 3.5–10 LKB ampholytes in the first dimension (IF) and 10% acrylamide/SDS in the second dimension (SDS). Gels *b* and *c* were loaded with 500,000 d.p.m. of labelled protein and exposed for 9 days; gel *a* was loaded with 5,800 d.p.m. of labelled protein and exposed for 7 days. All gels shown were processed for fluorography[17] and exposed to XS-1 film. Arrowheads indicate the position of the major PCNA spot. The actin (Ac), vimentin (V) and tubulin (T) spots are also indicated. Actin is precipitated nonspecifically by both anti-PCNA and control sera, in amounts that vary between experiments. The gel shown in *a* contains actin serving as internal marker.

Fig. 3 (Right) PCNA in normal and transformed rat cells and purified nuclei. Proteins of proliferating REF52 cells 3 days after plating (*a*), quiescent REF52 cells 8 days after plating (*b*) and dividing REF52-AG6 cells 3 days after plating (*c*) were labelled for 24 h with ^{35}S-methionine and resolved in two-dimensional gels as in Fig. 2. Nuclear proteins from REF52-WT6 cells labelled for 2 h with ^{35}S-methionine were resolved in a similar gel (*d*). Nuclei were prepared by the method of Fleischer and Kervina[18]. REF52-WT6 cells are SV40-transformed REF52 cells[11], and REF52-AG6 cells are REF52-WT6 cells selected for growth in soft agar. Approximately 300,000 d.p.m. of labelled protein were applied to each gel. Gels *a*–*c* were exposed for 17 days and gel *d* for 4 days. The proteins indicated are the major PCNA spot (large arrowheads), actin (Ac), vimentin (V), tubulin (T) and tropomyosins 1–6 (numbers in *a*). The tropomyosin forms and their changes on transformation have been described elsewhere[12,19,20]. Quantitation was carried out using the QUEST system for computerized analysis of two-dimensional gels[21]. Each film was scanned using an Optronics P-1000 densitometer, and the spots were detected and integrated by the image analysis programs. Each film was calibrated[22] so that measurements of film density could be converted to d.p.m. per unit area before spot integration. The integrated d.p.m. for each spot was divided by the total trichloroacetic acid-precipitable d.p.m. applied to the gel. For the protein samples resolved in *a*–*c*, the proportion of total applied d.p.m. found in the PCNA spots was 0.014%, 0.008% and 0.067%, respectively.



but it was not a major protein constituent. It was also detectable in a resting culture of normal REF52 cells (Fig. 3*b*), although its level at quiescence was about half that in dividing cells. On the other hand, subconfluent SV40-transformed cells (Fig. 3*c*) contained about fivefold more PCNA than normal dividing REF52 cells (Fig. 3*a*). The magnitude of this increase, one of the largest inductions seen on transformation of REF52 cells by SV40 (data not shown), makes it unlikely that the higher level of PCNA in the transformed cells is due simply to their slightly shorter generation time. Furthermore, pulse-labelling experiments show that the rate of PCNA synthesis is elevated in all transformed lines examined, including cells transformed by SV40, adenovirus type 5 and Kirsten murine sarcoma virus[12].

In both HeLa cells (not shown) and SV40-transformed REF52 cells (Fig. 3*d*), PCNA was a major component of the nuclear fraction. It was more prominent in the nuclear pattern than in the whole cell protein pattern (Fig. 3*c*), unlike cytoplasmic components such as the tubulins and vimentin which were detectable but not enriched in the nuclei. However, the enrichment of PCNA in the nuclear fraction was not as great as that seen for certain other nuclear proteins. Indeed, some PCNA was detected in the cytoplasmic fraction of these cells (not shown), but in view of the overwhelmingly nuclear staining seen by immunofluorescence[2,3] this may represent a partial leaching from the nucleus during cell fractionation rather than a distribution between the two cellular compartments *in vivo*. Like its human counterpart, the rat PCNA spot was accompanied by a more acidic satellite spot. The ratio of the two forms did not vary substantially as a function of growth rate or subcellular fractionation, and the significance of this minor form is unclear.

These results demonstrate that PCNA is identical with cyclin in subcellular localization, electrophoretic behaviour and

peptide composition, as well as in biological properties. We suggest that the name PCNA be used in future, as it is the earlier nomenclature and the term cyclin has recently become ambiguous[13]. As shown here and elsewhere[3–9,12], the level of the protein falls in resting cells and, conversely, it becomes particularly prominent in transformed and tumour cells. Fluctuating PCNA levels have probably also been observed in other studies of transformed[14] and serum-stimulated[15] cells, but in these cases definite identification of PCNA is lacking. Although its function remains obscure, correlation of the previously unlinked reports on this transformation-sensitive and cell cycle-associated nuclear protein may yield illuminating clues. In this regard, it is intriguing that the maximal synthesis of PCNA appears to coincide with its accumulation in the nucleolus, preceding the peak of DNA synthesis[3,7]. The availability of relatively monospecific antibodies to PCNA should facilitate exploration of its connection with the nucleolus, cell division and transformation.

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Synthesis of biologically active rat transforming growth factor I

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The hypothesis that some transformed cells produce endogenous transforming growth factors (TGFs) has been supported by isolation of peptide factors from transformed cells[1–5]. One group of TGFs, TGF-I, produced only by transformed cells, displays sequence homology with the functionally related mouse submaxillary epidermal growth factor (mEGF)[5]. Both TGF-I and mEGF exhibit similar activities in competition for binding to the EGF receptor, stimulation of DNA synthesis and cell growth. Another group of TGFs, TGF-II (also known as TGF_β)[2], present both in normal and transformed cells, is structurally and functionally unrelated to TGF-I or EGF, and does not compete for binding to the EGF receptor or induce cell growth. However, TGF-I or EGF in the presence of TGF-II produces a synergistic effect that is responsible for the observed phenotypic transformation of NRK fibroblasts[4]. The complete amino acid sequence of rat TGF-I (rTGF-I) from transformed Fischer rat embryo fibroblasts, has recently been determined[5]. Using this proposed sequence, we have now prepared synthetic rTGF-I by the solid-phase synthesis method and find that it exhibits chemical and biological properties indistinguishable from those of natural rTGF-I. Since synthetic rTGF-I is free of any biological contaminants, our findings provide independent evidence that rTGF-I is an active principle in the transformation of cells[6].

Rat TGF-I purified from the conditioned medium of Fisher rat embryo fibroblasts transformed by feline sarcoma virus is a single peptide chain containing 50 amino acid residues and 3 disulphide linkages. The primary structure was determined by Edman degradation in a gas-phase microsequencer to be[5]: H-Val-Val-Ser-His-Phe-Asn-Lys-Cys-Pro-Asp-Ser-His-Thr-Gln-Tyr-Cys-Phe-His-Gly-Thr-Cys-Arg-Phe-Leu-Val-Gln-Glu-Glu-Lys-Pro-Ala-Cys-Val-Cys-His-Ser-Gly-Tyr-Val-Gly-Val-Arg-Cys-Glu-His-Ala-Asp-Leu-Leu-Ala-OH.

The chemical synthesis of rTGF-I was performed manually by the stepwise solid-phase approach, following the general principles described by Merrifield[7]. We adopted the differential acid-labile protecting group strategy, using the conventional combination of t-butyloxycarbonyl for the N^α-amino terminus and benzyl alcohol derivatives for the side chains. An improved, more acid-stable benzyl ester linkage that anchored protected amino acids to the polymeric support was used to minimize loss of peptides during the repeated acid treatments[8]. Complete deprotection and removal of peptide from the resin was achieved by the new low–high HF method[9,10], which removed benzyl protecting groups by the S_N2 mechanism in dilute HF solution; this minimizes the serious side reactions due to carbocations generated in the conventional S_N1 deprotection method. It also minimizes the cysteinyl side reactions that can hamper the synthesis of proteins containing multiple disulphide linkages.

After HF treatment and before any purification, the crude and reduced synthetic rTGF-I was oxidized and regenerated by the mixed disulphide method in the presence of a combination of reduced and oxidized glutathione[11]. This avoided the formation of polymeric materials during purification. The regenerated, crude rTGF-I contained 40–50% of EGF radioreceptor and tyrosine-specific protein kinase activities when compared with natural rTGF-I. Crude synthetic rTGF-I was purified to homogeneity in three steps: (1) gel filtration on a BioGel P-10 column, (2) ion-exchange chromatography on a CM-Sephadex column and (3) preparative HPLC on a C_{18} reverse-phase column. Overall yield, based on starting loading of Ala to resin, was 31%.

In both reducing and nonreducing conditions, SDS-polyacrylamide gel electrophoresis of the purified synthetic rTGF-I gave a single band with an apparent molecular weight of 7,000. Amino acid analysis by 6M HCl and enzymatic hydrolysis provided the expected theoretical molar ratio of the proposed sequence. No free thiol was detected in synthetic rTGF-I by Ellman's method of sulphydryl determination[12], but thiolytic reduction produced the expected theoretical value of six cysteine residues. These findings support the conclusion that synthetic rTGF-I is a single-chain polypeptide containing six cysteines in disulphide linkages, which agrees with the expected chemical properties of natural rTGF-I. More importantly, synthetic rTGF-I co-eluted

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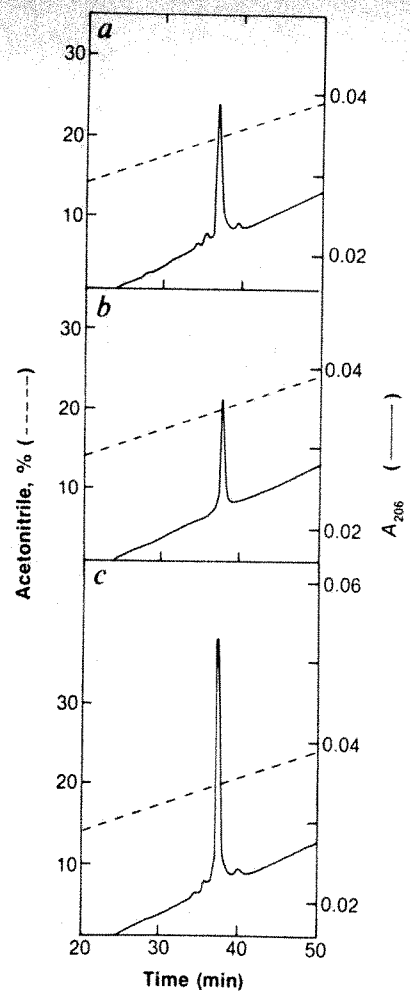


Fig. 1 HPLC of synthetic and natural rat TGF-I. *a*, 33.3 EGF-equivalents of natural rTGF-I; *b*, 26.3 EGF-equivalents of synthetic rTGF-I; *c*, 33.3 EGF-equivalents of natural rTGF-I and 26.3 EGF-equivalents of synthetic rTGF-I. Unit EGF equivalency is based on the displacement of ^{125}I-EGF in the radioreceptor assay. **Methods:** Crude synthetic rTGF-I was batch purified by gel filtration (P-10 column, 2.5 × 75 cm, elution with 1 M acetic acid), ion-exchange chromatography (CM-Sephadex column, 2.5 × 15 cm, linear *p*H gradient of 5.6–8.0 in 0.1 M NH_4OAc) and finally preparative HPLC (2.5 × 30 cm at a flow rate of 1.4 ml min^{-1}, eluting with a linear gradient of acetonitrile in 0.033% trifluoroacetic acid). Analytical HPLC was performed on a μBondapack C_{18} column (10 μm particle size, 0.39 × 30 cm, Waters Associates) at a flow rate of 1 ml min^{-1} at 40 °C. Peptides were eluted with a linear gradient of acetonitrile in 0.045% trifluoroacetic acid and were monitored at 206 nm.

with natural rTGF-I as a single symmetrical peak in C_{18} reverse-phase HPLC (Fig. 1).

Synthetic rTGF-I was compared with natural rTGF-I in three assays for the biological properties of the putative transforming growth factor. In the mitogen assay, TGF-I-stimulated growth of serum-deprived normal rat kidney cells was measured by the incorporation of ^{125}I-iododeoxyuridine. The soft agar assay, performed in the presence of fetal bovine serum and TGF-II, confirmed that the morphological and phenotypic alterations induced by rTGF-I could be quantified by colony formation in soft agar. The latter assay has been shown to correlate well with tumorigenicity[13]. Fetal bovine serum or TGF-II alone does not induce transformation of NRK cells in culture. Similarly, neither natural nor synthetic TGF-I produces such an effect in the absence of TGF-II. Both synthetic and natural rTGF-I displayed similar dose–response curves and half-maximal activities in these two assays (Fig. 2).

As rTGF-I is structurally related to EGF[14,15] and competes with mEGF for the binding of EGF receptors on cellular membranes[1], synthetic rTGF-I was compared with natural rTGF-I for binding on A431 human carcinoma cells. Again, the response and activities of the natural and synthetic rTGF-I were indistinguishable from each other (Fig. 3). The concentration required for 50% inhibition of ^{125}I-EGF binding was 3.5 and 4.1 nM for natural and synthetic rTGF-I, respectively. A consequence of TGF or EGF binding to the EGF membrane receptors is the stimulation of phosphorylation of tyrosine residues in synthetic peptides or endogenous substrates[16,17]. Synthetic rTGF-I was found to stimulate the phosphorylation of the synthetic angiotensinyl peptide substrate[18] with a half-maximal activity

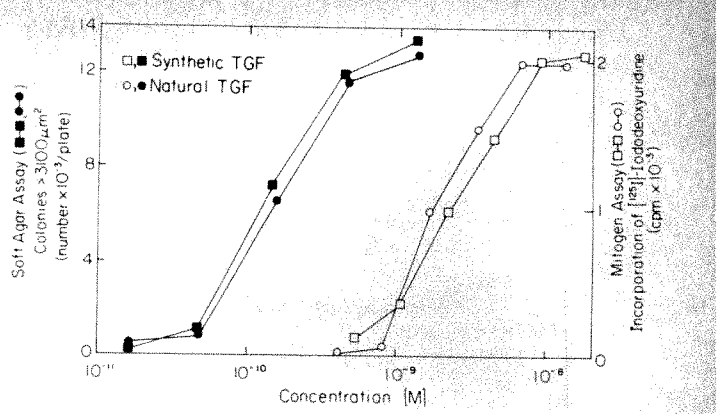


Fig. 2 Dose–response curves of mitogen and soft agar growth assays of synthetic and natural rTGF-I on normal rat kidney cells. The mitogen assay (open circles and squares) was performed in flat-bottom microwells (1 × 10^4 cells per well) in 100 μl Dulbecco's minimal essential medium with 10% heat-inactivated fetal bovine serum for 24 h and maintained in medium containing 0.1% fetal bovine serum for 7 days before the assay. Test samples were kept in 50 μl Waymouth medium[19], and [5-^{125}I]iodo-2′-deoxyuridine (^{125}I-IUdR, 2.5 μCi ml^{-1}, Amersham) was added for the final 24 h of culture[20]. Cells were washed and collected using a multiple harvester and counted in a γ-counter. Maximal ^{125}I-IUdR incorporation (2,048 c.p.m.) was 14-fold above control values (144 c.p.m.) without added mitogen. Values were corrected by subtracting ^{125}-I-IUdR incorporated into unstimulated control cultures. The assay for colony growth in soft agar (solid circles and squares)[21] using 49F fibroblast cells was performed as reported elsewhere[22], except that the crude platelet-derived murine TGF-II was added from stocks of 1 mg ml^{-1} in phosphate-buffered saline, containing 1 mg ml^{-1} bovine serum albumin. Stained colonies were quantified by number and size using a Bausch and Lomb image analyser[23]. The number of soft agar colonies (>3,100 μm^{-2}) in the presence of TGF-II was 96 per plate and was subtracted.

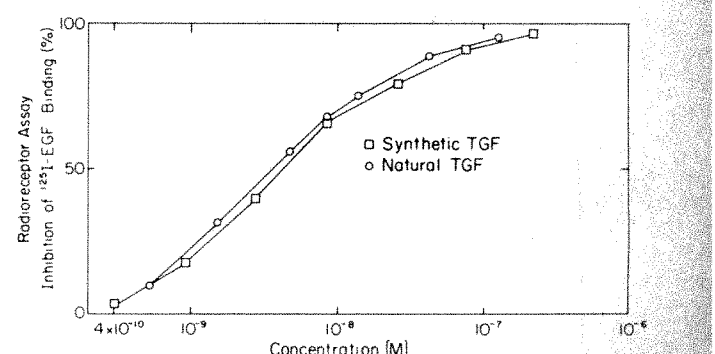


Fig. 3 Dose–response curve of radioreceptor assay of synthetic and natural rTGF-I. Purified mEFG was radiolabelled with Na ^{125}I by a modified chloramine-T method as described previously[5]. The ^{125}I-mEGF binding assay[1] was performed on subconfluent monolayers of formalin-fixed A431 cells[1]. Cells (8 × 10^3 per dish) were incubated with ^{125}I-mEGF (0.8 ng ml^{-1}, 82 μCi per μg) and unlabelled peptides at the indicated concentrations. The concentrations of natural and synthetic rTGF were quantified by amino acid analysis of companion aliquots. The ^{125}I-mEGF bound was determined, and nonspecific binding (binding in the presence of 2 μg ml^{-1} mEGF) was 2.4% and was subtracted. 100% binding corresponded to 10% of the input radioactivity.

of 0.3 nM (data not shown), comparable with the reported activity for natural rTGF-I[16].

In conclusion, we have demonstrated that synthetic rTGF-I possesses chemical and biological properties indistinguishable from those of natural rTGF-I. These findings, therefore, provide convincing evidence that the chemical structure of rat TGF-I is that proposed by sequencing studies[5]. Since synthetic rTGF-I is completely free of biological contaminants, its biological properties must be entirely due to the proposed structure of rat TGF-I, confirming the view that transformed cells produce endogenous growth stimulating polypeptide factors.

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Reversible inhibition of translation by *Xenopus* oocyte-specific proteins

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A characteristic of growing oocytes of all animal species is the synthesis and accumulation of messenger RNA which is destined to be used primarily by the early embryo[1,2]. The mechanism(s) which regulates the translation of this maternal mRNA remains unknown. However, the inability of the oocyte to translate all of its putative mRNA has been attributed to at least three limitations: (1) The rate of translation is limited by the availability of components of the translational apparatus other than mRNA[3,4], (2) the structural organization of the mRNA prevents translation[5,6], and (3) proteins associated with the mRNA prevent translation[7,8]. Several investigators have suggested that proteins associated with maternal mRNA suppress translation in sea urchin eggs[7–10], although others claim that such results may be due to experimental artefacts[11,12]. Oocyte-specific proteins have been identified in association with non-translating poly(A)$^+$ mRNAs from *Xenopus laevis* oocytes[13–16], and we report here that when these proteins are reconstituted with mRNAs *in vitro* the translation of the mRNAs *in vitro* is reversibly repressed. The implication is that these proteins are involved in the regulation of translation of stored maternal mRNAs.

Recent studies have demonstrated that RNAs and proteins may be reconstituted faithfully *in vitro* (for example, refs 17–19).

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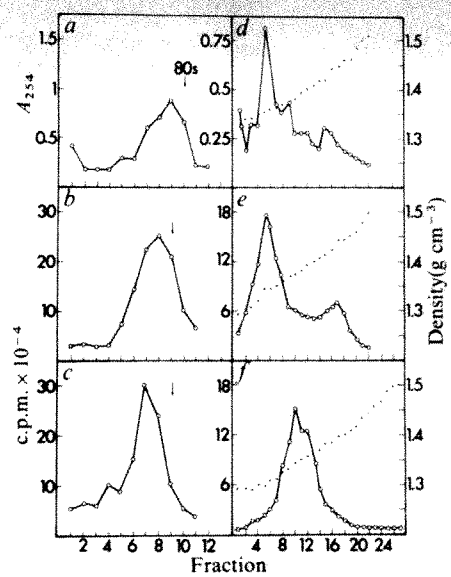


Fig. 1 Centrifugation of native and reconstituted RNPs. Formaldehyde-fixed native RNPs from oocytes were centrifuged through a sucrose gradient, fractionated and the absorbance at 254 nm in each fraction was determined (*a*). The fractions corresponding to 40–60S (fractions 8, 9) were collected, dialysed and centrifuged through a CsCl gradient. Fractions (0.2 ml) were collected and absorbance at 254 nm was determined, as was the density which was calculated from refractive index (*d*). Iodinated poly(A) RNA from stage 1–2 oocytes and rabbit globin mRNA were reconstituted with the RNA binding proteins and were centrifuged through sucrose gradients (poly(A) RNP (*b*); globin mRNP (*c*)). The 40–60S fractions from these gradients were then collected, fixed and centrifuged on CsCl gradients (poly(A) RNP (*e*); globin RNP (*f*)). Values for radioactivity (○–○) and density (···) are shown. The arrow indicates where the 80S monosome sedimented.

Methods: Stage 1–2 *Xenopus* oocytes were homogenized in polysome buffer (0.3 M KCl, 2 mM $MgCl_2$, 20 mM Tris-HCl *p*H 7.4, 4 μg ml^{-1} polyvinyl sulphate, 0.01% diethylpyrocarbonate, 10 μg ml^{-1} cycloheximide and 0.5% Nonidet P-40) and the post-mitochondrial supernatant was then centrifuged through a 5–30% sucrose gradient in buffer in an SW41 tube for 3 h at 4 °C at 34,000 r.p.m. The gradient fractions corresponding to the 40–60S region were collected, dialysed against 10 mM phosphate buffer *p*H 7.2, and then fixed in phosphate-buffered 4% formaldehyde for 2 h at 4 °C[27]. These particles then were suspended in 10 mM HEPES *p*H 7.5 and 0.5 M NaCl and applied to an oligo-d(T) cellulose column (P-L Biochemicals, type 7). The column was washed in high salt buffer until no UV(A_{254})-absorbing material was detected in the eluate. The poly(A) RNPs were then eluted in 10 mM HEPES *p*H 7.5. Fixed RNPs were centrifuged through preformed 30–60% CcCl gradients[28] for 12 h in an SW 50.1 tube at 33,000 r.p.m. at 4 °C. Poly(A) RNA and globin mRNA were iodinated by the $TlCl_3$ method as described previously[6,16].

To obtain oocyte protein for reconstitution experiments, stage 1–2 *Xenopus* oocytes were homogenized and the ribonucleoprotein (RNP) particles sedimenting through a sucrose gradient to a 40–60S fraction were collected. The proteins from such RNPs were purified further by elution from a phosphocellulose column in high salt as reported previously[16]. The five major proteins in this fraction have molecular weights of 50,000–94,000, bind poly(A)$^+$ RNA with high affinity and are oocyte-specific[16]. For reconstitution studies, these proteins were mixed with ^{125}I-labelled poly(A) RNA from stage 1–2 oocytes and compared with native particles by sucrose gradient and CsCl gradient centrifugation. Reconstituted poly(A) RNP particles sedimented primarily at 40–60S (Fig. 1*b*), as did the native particles originally used to obtain the proteins used for reconstitution (Fig. 1*a*). Most of the reconstituted poly(A) RNP particles which sedimented at 40–60S (Fig. 1*b*) exhibited a buoyant density in CsCl of 1.34 g cm^{-3}, with a minor peak at a density of 1.44 g cm^{-3} (Fig. 1*e*). These values again are virtually identical with those obtained with native particles which exhibited a peak

Table 1 Relative translatability of reconstituted globin mRNA in injected oocytes

	Synthesis of globin relative to injected pure globin mRNA (%)						
	Expt no.						
	1	2	3	4	5	6	$x \pm$ s.d.
Pure globin mRNA	100	100	100	100	100	100	100 ± 0
Globin mRNA + histones	140	139	133	111	—	—	131 ± 12
Globin mRNA + ribosomal proteins	—	150	155	120	—	—	142 ± 15
Globin mRNA + basic liver proteins	—	105	137	101	—	—	114 ± 16
Globin mRNA + basic leg muscle proteins	—	—	—	—	—	97	97
Globin mRNA + basic heart proteins	—	—	—	—	—	96	96
Globin mRNA + oocyte RNA binding proteins	5	30	37	11	40	37	25 ± 17

Expt 1: Four stage VI *Xenopus* oocytes were injected with 10 ng of pure globin mRNA, 10 ng of globin mRNA reconstituted with 10 ng calf thymus histones, or 10 ng of oocyte binding proteins (preparation 1) prepared as follows. Stage 1–2 *Xenopus* oocytes were homogenized and the post-mitochondrial supernatant was centrifuged through a sucrose gradient[16]. The RNPs sedimenting to the 40–60S fraction were collected and applied to a phosphocellulose column. The proteins eluting in 1–2 M NaCl were used for reconstitution[16]. Reconstitution conditions were 30–60 min on ice in 10 mM Tris-HCl *p*H 7.5 and 50 mM NaCl. Oocytes were cultured for 12 h in OR-2 medium[24] and then injected with ^{3}H-leucine[4]. Oocytes were then cultured for 1 h. The radioactive proteins were collected[4], electrophoresed on a SDS-12.5% polyacrylamide gel and the radioactive globin band was cut out, oxidized and the radioactivity determined by liquid scintillation spectrometry[4]. **Expts 2–6:** Five oocytes were injected with 3 ng pure globin mRNA, or globin mRNA reconstituted with 30 ng of calf thymus histones, *Xenopus* oocyte ribosomal proteins (that is, *Xenopus* stage VI oocyte proteins extracted from RNP particles with high salt (2 M NaCl) which sedimented at 80S and had a molecular weight <60,000 as determined by gel filtration), *Xenopus* liver, leg muscle and heart proteins prepared in the same way as the oocyte RNA binding proteins[16], or *Xenopus* stage 1–2 oocyte RNA binding proteins[16]. Oocytes were cultured for 18 h in modified Barth's saline[25] and then injected with ^{35}S-methionine[26]. Oocytes were cultured for an additional hour and the protein prepared as described previously[4]. The relative amount of globin synthesis was determined by densitometry of autoradiograms or by direct quantitation of the radioactivity in the putative globin band. Expt 2 used oocyte RNA binding protein preparation number 2, expt 3 used preparation 3, expt 4 used preparation 4 and expt 5 used preparation 5. The autoradiogram shown in Fig. 2 was taken from expt 4.

density of 1.36 g cm^{-3}, with a minor peak at 1.42 g cm^{-3} (Fig. 1*d*). Similar experiments were performed with ^{125}I-labelled rabbit globin mRNA. Reconstituted globin mRNP also sedimented in the 40–60S region of the gradient (Fig. 1*c*) and had a peak buoyant density of 1.34 g cm^{-3} (Fig. 1*f*). Using the formula of Spirin[20], the native RNPs had a protein to RNA mass ratio of 4.5 : 1, very similar to a previous estimate of oocyte poly(A) RNP protein to RNA mass ratio of 4 : 1 (ref. 14). The reconstituted poly(A) RNP and globin RNP had protein to RNA mass ratios of 5.5 : 1. Other experiments (data not shown) demonstrated that that same ratio was obtained even with a 10-fold greater amount of protein in the initial incubation with the RNA. Thus, based on the physical characteristic we have measured, the reconstituted RNPs appear to be very similar to native RNPs.

To test the translatability of reconstituted RNPs *in vitro*, we have injected RNPs into *Xenopus* oocytes. Since the endogenous mRNAs being translated represent a subset of total poly(A) RNA in the oocyte, the translation of reconstituted poly(A) RNAs would be difficult to assess quantitatively[6]. Accordingly, we have determined whether the oocyte-specific RNA binding proteins affect the translation of globin mRNA. Figure 2 shows an SDS gel autoradiogram of the proteins synthesized in mRNP-injected oocytes. When pure globin mRNA was injected, globin synthesis was readily detected (Fig. 2, lane 2). However, when globin mRNA was reconstituted with oocyte proteins before injection, no globin synthesis was apparent (lane 3). Globin mRNA reconstituted with other basic proteins such as histone (lane 4) or ribosomal proteins (lane 5) was still translatable. Furthermore, reconstitution of globin mRNA with basic liver proteins (lane 6), extracted from the 40–60S particle fraction and purified as described for the oocyte proteins, did not prevent translation.

Table 1 summarizes several experiments to determine the amount of globin synthesized as a consequence of reconstitution with protein from several sources. The reconstitution of globin mRNA with histones or ribosomal proteins or proteins from frog liver, muscle or heart prepared in a manner identical with the oocyte proteins described above, results in as much as 42% enhancement of globin synthesis. Examination of the protein species in these various fractions reveals that those present in the oocyte are absent from the somatic tissues (data not shown; see also ref. 16). As most globin mRNA turns over following injection[4], it is likely that the non-oocyte proteins protect the mRNA from oocyte nucleases until it is loaded onto polysomes. As Fig. 2 indicates, the oocyte-specific proteins inhibit globin synthesis in injected oocytes. On average, one-quarter as much globin is synthesized when the mRNA is reconstituted with the oocyte proteins as when pure mRNA is injected. Although there was some variation between preparations, the protein always inhibited globin mRNA translation by at least 60%.

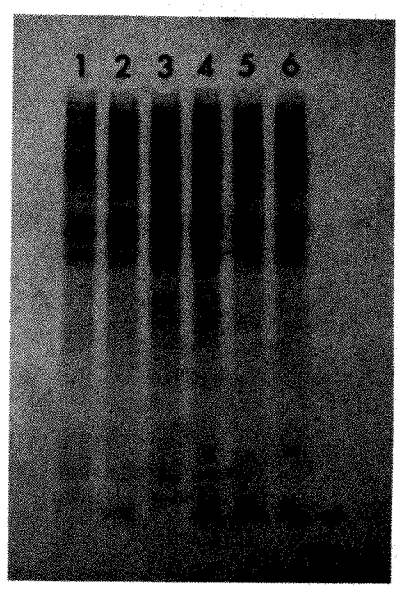


Fig. 2 Autoradiogram of proteins synthesized in mRNP-injected oocytes. Oocytes were injected with 5 ng of pure rabbit globin mRNA (lane 2), or 5 ng globin mRNA reconstituted with 30 ng of stage 1–2 oocyte proteins (lane 3), 30 ng of histones (lane 4), 30 ng of ribosomal proteins (lane 5), or 30 ng basic liver proteins (lane 6). Control oocytes were not injected (lane 1).
Methods: Conditions for reconstitution are described in Table 1 legend. Oocytes were injected with mRNA or mRNP, cultured for 18 h, injected with ^{35}S-methionine (Amersham; 1,220 Ci mmol^{-1}) and cultured for an additional hour. Oocytes were homogenized in 50 mM NaCl, the protein was collected[4], and resolved by SDS-12.5% polyacrylamide gel electrophoresis.

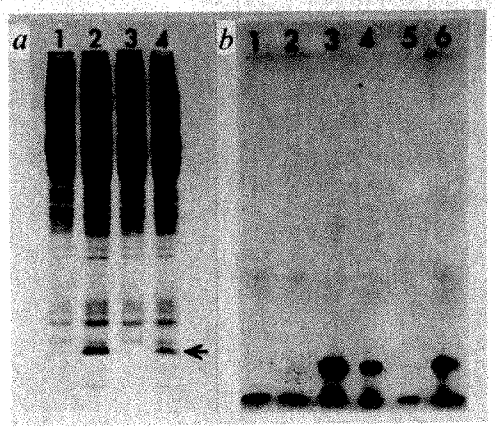


Fig. 3 The inhibition of translation by oocyte-specific proteins is reversible. *a*, Five noninjected oocytes (lane 1), or oocytes injected with 5 ng pure globin mRNA (lane 2), 5 ng globin mRNA reconstituted with oocyte proteins (lane 3), or 5 ng globin mRNA reconstituted with oocyte proteins and then deproteinized (lane 4), were cultured and then injected with ^{35}S-methionine. The resulting radioactive proteins were resolved by SDS-gel electrophoresis and fluorography. *b*, Synthesis of reconstituted globin mRNA in a wheat germ lysate. Lane 1, no mRNA added; lane 2, 1 μg globin mRNA reconstituted with 1 μg of oocyte proteins and then digested with 1 μg ml^{-1} RNase A; lane 3, 1 μg pure globin mRNA; lane 4, 1 μg globin mRNA reconstituted with 1 μg oocyte protein; lane 5, 1 μg globin mRNA reconstituted with 9 μg oocyte protein; lane 6, 1 μg globin mRNA reconstituted with 9 μg oocyte protein and then deproteinized.

Methods: Globin mRNA and oocyte proteins were reconstituted as described in Table 1 legend (expt 4). One-half of the mixture (as well as pure RNA) was extracted once with buffer(10 mM Tris-HCl *p*H, 0.1% SDS)-saturated phenol/chloroform/isoamyl alcohol (25:24:1) and once with chloroform/isoamyl alcohol (99:1). The aqueous phases were then injected without further modification. The efficiency of recovery was ~80% based on the extraction of reconstituted ^{125}I-globin mRNA. Following RNP or RNA injection, oocytes were cultured for 18 h and then injected with ^{35}S-methionine. The resulting radioactive proteins were collected and analysed as described for Fig. 2. The wheat germ lysate (BRL) reaction volume was 30 μl containing 5 μCi of ^{35}S-methionine. The reaction was carried out at 25 °C for 1 h. Deproteinization and the analysis of the radioactive proteins were as described above.

We have assayed for ribonuclease contamination in the oocyte protein preparation. ^{32}P-labelled total oocyte RNA was mixed with the oocyte proteins and the RNA was resolved by denaturing agarose gel electrophoresis and autoradiography. In contrast to pure ^{32}P-labelled oocyte RNA, no degradation of the reconstituted RNA was observed (data not shown). In addition, total RNA from oocytes injected with globin mRNA reconstituted with the oocyte proteins was extracted and used for an RNA gel blot. Using ^{32}P-labelled globin cDNA as the probe, only full-size globin mRNA was evident when pure or reconstituted globin mRNA was injected (data not shown). However, the possibility remained that the oocyte proteins rendered the mRNA nontranslatable by some permanent modification not detected by the methods described above. Therefore, we determined whether reconstituted globin mRNA may become translatable following deproteinization. Figure 3*a* again demonstrates that globin synthesis was detected when pure mRNA (lane 2), but not reconstituted mRNA (lane 3), was injected into oocytes. When reconstituted mRNA was deproteinized by phenol and chloroform and then injected, globin synthesis was evident (lane 4). The synthesis of globin in oocytes injected with deproteinized reconstituted globin mRNA was always 20–40% lower than when the pure mRNA was injected. This difference cannot be accounted for by a very low recovery of the mRNA (see Fig. 3 legend) and possibly results from incomplete deproteinization. Nonetheless, we do conclude that the oocyte proteins reversibly inhibit the translation of injected mRNA in injected oocytes.

The ability of the oocyte proteins to inhibit translation of globin mRNA *in vitro* was also tested (Fig. 3*b*). Whereas the translation of globin mRNA was very efficient in a wheat germ lysate (lane 3), that of mRNA reconstituted with increasing amounts of the oocyte proteins became progressively more inefficient (lanes 4, 5).

We have conducted preliminary experiments which suggest that mRNP unmasking can occur *in vivo*. These experiments are based on the observation that in oocytes induced to undergo maturation by progesterone, general protein synthesis approximately doubles[21] due to the recruitment of previously untranslated mRNA onto polysomes[22]. If this recruitment involves mRNP unmasking, it might be expected that injected globin mRNP would become translatable in the mature oocyte. When such experiments were done in oocytes injected with reconstituted globin mRNP and then induced to mature, there was a 3.5-fold increase in radioactivity in the globin band. However, in noninjected oocytes which had undergone maturation, a 2.9-fold increase in radioactivity occurred in the region of an SDS gel where globin would be expected to migrate. Although we tentatively attribute this difference to globin mRNP unmasking, the fact that the synthesis of endogenous proteins partially obscures our quantitation of globin synthesis suggests the need for a more rigorous study to confirm these observations.

The present study demonstrates that oocyte-specific proteins extractable from native RNPs may be reconstituted with mRNAs *in vitro* to form particles which resemble native RNPs. Further, these proteins can reversibly repress the translation of the mRNA *in vivo* (as well as *in vitro*). Several control experiments support the view that this result is not due to nonspecific inhibition. Thus, we now have a system whereby proteins can be used in reconstitution experiments and their ability to alter translation and/or stability of mRNA in *in vivo* conditions may be investigated. In this connection, a monoclonal antibody to an oocyte RNA binding protein has been isolated[23]. Thus, with this and other monoclonal antibodies, individual proteins may be isolated and reconstituted with mRNA, and the function of each assessed.

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French change

Guy Ourisson

Pour Sauver l'Université. By Laurent Schwartz.
Editions du Seuil, 27 rue Jacob, F-75261, Paris Cedex 06: 1983. Pp.123. Pbk FF 49.

LAURENT Schwartz, a Fields medallist, is certainly the most outspoken mathematician in France. For years, he has persistently taken strong public stands against colonialism in Algeria, for human rights and civic liberties in the USSR, for progress through education and scientific research, and for the electoral victory of the Left in France. So when he writes of the reforms currently underway in French universities that "mediocrity will take precedence over quality, diversity will disappear into drabness, responsible decisions will be made impossible by irresponsible politicking", everybody in French academic circles is likely to listen to what he has to say.

Schwartz sets the tone unambiguously: "I hope the present socialist experiment will succeed. I want to help the Government, and this is why I criticize it". Despite this clear warning, I had expected, when this book appeared last autumn, that his analysis — expert, explicit and caustic — would become political TNT graciously handed to the opposition parties. This, in fact, has not really been the case; when the book came out, the opposition had already shifted its attention to more popular disputes than the Savary reform. Such is the prodigious capacity of Gallic organizations to lose interest once a law has been passed and all that remains to do is to try to apply it.

Pour Sauver l'Université has now been read by everybody in French academic circles; at least, the reviews have been read. The book has helped to focus the final round of discussions in Parliament on some of the key points of the Savary reform. It has helped in generating, in the universities, new trends and new view points. It has been a useful book, but one which has in part estranged Schwartz's own Left, the author being branded as an elitist, an arch-enemy of the Unions and the avowed leader of the "mandarins" (in France, the reactionary professors). Schwartz's influence has rallied around the slogan "Qualité de la science" lists of candidates, politically non-aligned and independent of the trade unions, in recent elections to various national "Comités" — and his name has helped these candidates to be elected *en masse*. So the impact of this little book in France has been great. But who outside of France would discover it to be worth reading?

What is found in *Pour Sauver l'Université* ranges from the specific to the general. I doubt if anybody outside France, except perhaps students of French education, would bear reading the chapter criticizing the new voting procedures chosen for the various bodies of university self-government (those same procedures which a few months later enabled "Qualité de la science" candidates to be elected in great numbers). I also doubt if anybody outside the French system would gain anything useful from the chapter describing the dual higher-education system in France, in which Grandes Ecoles thrive alongside universities, select supposedly the best students, in some areas monopolize the job market, nurture the industrial elite, and yet are in many cases appallingly alien to the very notions of innovation and of research. We French have to cope with this system, and try to adapt it to changing needs — but nobody else would gain from adopting it.

On the other hand, it is quite probable that a longer lasting interest could be found in the three remaining chapters: two on the virtues of selection of students, and on the way to organize it, and the last one on the interaction between teaching and research. These are real, general problems, common to many countries, and Schwartz has a very wide knowledge of the situation in the United States, in Great Britain and in Germany. His opinions therefore carry some weight. For instance, he gives a very lucid discussion of the fallacy equating free entry into universities with the democratization of higher education — particularly when students, once admitted, are not orientated towards fields adapted to their skills and thus in part towards the job market, but have to cope unassisted with examiners determined to let only the fittest survive. He also broadly defines conditions which would favour a genuine democratization of the higher education system — by setting ambitious goals for students and helping them to choose progressively the area in which they could excel, or at least do their best. Admittedly, this is the very philosophy of the Savary reform; but here, as in many other places in the book, Schwartz is more critical of what he fears will be made of these changes, by us his colleagues, than of the content of the new law itself. Obviously, he shares with me a certain scepticism of the real efficiency, in France, of a "definitive" law.



Students march through Paris in May last year in protest at the proposed reform of the nation's university system.

For foreign readers, however, probably the most informative chapter is the one devoted to research (more precisely, to remain in tune with the book's general theme, to the "Dangers Looming over Research"). This gives a good description of what really works well in French research (apart from Big Science): the unique symbiosis of the National Research Council (the Centre National de la Recherche Scientifique) and the universities, which might well be a worthwhile export item. Schwartz believes it is endangered; I do not, and I do not believe that any law can threaten it more than our natural tendency to divide Gallia into as many little fiefdoms as we can.

Anybody interested in understanding the currents now flowing in French academia, and shaping it anew, should read this short and intelligent book. A complementary audience might also include students of academic research as well as some sociologists and historians. But whatever their background, all readers should remember to place Schwartz's thesis within a general perspective of the French political situation in which it is rooted. □

Guy Ourisson is Professor of Chemistry at the Université Louis Pasteur in Strasbourg. From 1970 to 1975 he was President of the Université Louis Pasteur, and in 1981–1982 was Director-General of Higher Education and Research responsible to M. Savary.

ADVERTISEMENT

The current issue of *Peptides*: vol 5, no. 2 (March/April), 1984, is devoted to recent progres in research on vaso-active intestinal polypeptide (VIP) and related peptides. The papers, based on presentations at the 1st international symposium on VIP, held in Brussels, September 13-16, 1983, deal with aspects of chemistry, biochemistry, anatomic localization, physiology, pathophysiology and clinical significance of VIP (and to some extent, secretin & PHI). Topics relate to the neuroendocrine, gastrointestinal, cardiovascular, respiratory, and reproductive systems.

The journal may be ordered from: ANKHO International, Inc., P.O. Box 426, Fayetteville, NY 13066

Underwater illumination

M. J. Dring

Light and Photosynthesis in Aquatic Ecosystems.
By John T. O. Kirk.
Cambridge University Press: 1983.
Pp.401. £37.50, $74.50.

THERE are advantages and disadvantages in attempting to bridge the gap between two scientific subjects. Ideally, the bridge will provide a route for the two-way traffic of ideas and scientists; but if the two subjects are in very different states of development, it may result instead in a brain-drain from the weaker to the stronger subject.

By bringing together the physics of underwater light and the biology of aquatic photosynthesis, Dr Kirk's book seems more likely to produce the latter result. Part I ("The Underwater Light Field") constitutes such a superb manual for would-be modellers that it may well persuade many aquatic biologists that, given the availability of modern underwater irradiance meters and microcomputers, it is very much easier to measure light than to measure photosynthesis. Part II ("Photosynthesis in the Aquatic Environment"), on the other hand, creates the impression that biologists are still far from the position where they can make much use of the exact information about underwater light described in Part I. There is, therefore, little incentive for physicists to become involved with photosynthetic problems and to reciprocate the movement of biologists into their field. This is a familiar problem at the boundaries of biology and physics, but one which is accentuated to a possibly unnecessary degree by the contrast between the two parts of Dr Kirk's book.

Part I is rigorous, lucid and concise. A review of the optical properties of water leads into thorough accounts of what to measure and how to measure it, and how these measurements can be used to characterize fully the underwater light field. Biologists may wonder from time to time if they really need to know all of the details discussed, but Dr Kirk provides clear, complete answers to their commonest questions, together with valuable compilations of measurements from water bodies around the world. Here, at last, is the complete manual on underwater optics for biologists, which must become an essential reference for students of all photobiological processes underwater.

The excellence of Part I sets such a high standard that it may seem churlish to complain that Part II fails to live up to it. The problem lies partly in the nature of the subject matter — the physiology and ecology of diverse and variable organisms cannot be treated as rigorously as the physical properties of light and water — but also in the author's approach. Acknowledging that light is not the only controlling factor, he has attempted a reasonably comprehensive account of aquatic photosynthesis, in which few references to underwater optics are couched in more than general and qualitative terms. Consequently, those readers who know enough about aquatic photosynthesis to appreciate and benefit from Part I are liable to be disappointed by Part II. Instead of retracing the steps of other recent books and reviews, I feel that Dr Kirk should have tried to illustrate how our understanding of aquatic photosynthesis might be advanced by applying the knowledge of underwater light presented in Part I. Bridge builders must establish their bridgeheads before advancing into the hinterland. □

M. J. Dring is a Reader in Botany at the Queen's University of Belfast.

Seeing new light

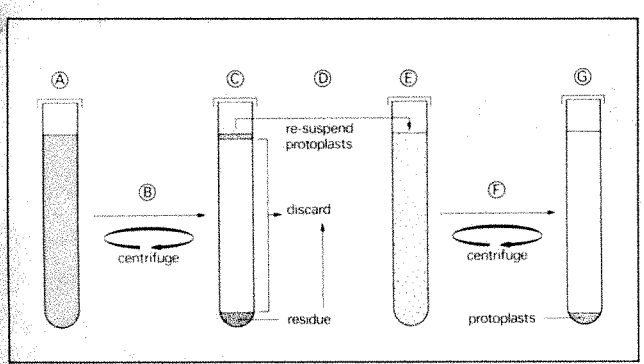
Alan E. Shapiro

Optics after Newton: Theories of Light in Britain and Ireland, 1704–1840.
By Geoffrey Cantor.
Manchester University Press: 1984.
Pp.257. £20, $25.

THE traditional history of optics from the publication of Newton's *Opticks* in 1704 to 1840 runs roughly as follows. The Newtonian emission or projectile theory of light dominated the wave theory in the eighteenth century, which, however, was overall a "sterile" period for optical research. The wave theory began to gain the upper hand at the beginning of the nineteenth century, when Thomas Young discovered the principle of interference, although initially his work was unjustifiably ignored. Finally, within a decade of Augustin Fresnel's comprehensive formulation of the wave theory (1815–1825), that theory vanquished its rival.

Geoffrey Cantor's primary aim in *Optics after Newton* is to reinterpret many elements of this story. He rejects the "sterility" thesis for the eighteenth century; he adds a third major theory of light, fluid theories, in which light is conceived to be a subtle fluid of interacting particles — not independent projectiles — flowing from a luminous source; and he argues that Young's principle of interference was rejected because it was perceived to be part of his otherwise unoriginal wave theory of light. I find Cantor's reinterpretation to be largely unconvincing. The primary cause of this is that the author is more concerned with historiography than with the history of optics, which he

The woof and the warp of tissue culture



Y. Y. Gleba, K. M. Sytnik

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Editor: **R. Shoeman**

1984. 62 figures. Approx. 245 pages
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The book contains many results not previously available in English. Originally published in Russian in 1982, it was revised considerably and up-dated for this English edition.

Petunia

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This book covers the classical aspects of *Petunia* biology as well as the more modern subjects of genetics and biochemistry of genes involved in floral pigmentation, molecular biology, haploidy, cell and anther culture, and protoplast fusion.

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T. L. Simpson

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1984. Approx. 220 figures. Approx. 585 pages
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J. Reinert, M. M. Yeoman

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uses to test various ideas about scientific communities, natural philosophy and scientific change.

Cantor takes a sociological approach. This is most fruitful in the treatment of the surprisingly large number of fluid theorists that he has uncovered, for they were for the most part attempting to use theories of light in Biblical interpretation and were not engaged in science, by making predictions, doing experiments or explicating optical phenomena. By Cantor's own account, they were marginal to the British scientific community at the beginning of his period of study and irrelevant by its end.

When Cantor turns to more serious scientific theories, such as the Newtonian emission theory, his sociological concepts are too narrowly conceived. For instance, he argues that no research occurred in the emission theory from 1700 to 1740, because its proponents were diverted by teaching. Besides the simple confusion of a cause and a correlation, this sort of claim could dily be tested by a comparison with the lation on the Continent during that period. Indeed, Cantor's self-imposed restriction to Britain and Ireland is a serious flaw, for a strong case can be made that the British Isles were a scientific backwater for most of the period from 1704 to 1840, so that what he has given us is actually an account of a relatively stagnant scientific community, rather than a progressive one. Thus, at the beginning of the nineteenth century, when he declares the emission theory to have been in decay in England, it was flourishing in France in the hands of Laplace, Biot and Malus.

An explanation for the initial neglect of Young's contributions — the most important and original British work in this study — provides, as Cantor recognizes, a critical test of his account. I am, however, only partly persuaded by his solution, namely, that the principle of interference was rejected because it was associated with Young's unoriginal wave theory. Cantor underestimates the quality of Young's work and depends too much on denigrating both him — Euler's "English disciple of moderate competence" — and his work, which he does not fully and clearly explain. He also introduces an artificial distinction that too sharply differentiates Young's and all earlier "vibration" theories from Fresnel's mathematical "wave" theory, whereas I believe there is a genuine continuity from Huygens through Euler and Young to Fresnel.

The most valuable feature of *Optics after Newton* is the vast quantity of primary literature described by Cantor, which can serve as a base for future work on the history of optics, not just British theories of light. □

Alan E. Shapiro is Associate Professor of History of Science and Technology at the University of Minnesota. He is the editor of The Optical Papers of Isaac Newton; *the first volume,* The Optical Lectures 1670–1672, *was recently published by Cambridge University Press.*

Firsts for feminism

Morris Kline

A Convergence of Lives: Sofia Kovalevskaia, Scientist, Writer, Revolutionary.
By Ann Hibner Koblitz.
Birkhäuser: 1984. Pp.305. $19.95.

WHY should anyone be interested in a biography of someone whose name, much less her work, is known to very few people and who died nearly one hundred years ago? The chief reason for this fine biography is that Sofia Kovalevskaia was a first-class mathematician, who achieved a place for herself in a period when women were hardly recognized outside the home.

In her time, Kovalevskaia achieved high professional status; she was the first woman to be awarded a doctorate in mathematics, the first (outside Italy) to become a professor of mathematics, and the first to serve as editor of a major mathematical journal. She also made impressive contributions to literature, and engaged in social and political efforts in the cause of women's rights. All of these activities were carried on during a life span of only 41 years (1850–1891) and despite the inevitable opposition from male professors in mathematics and other fields.

Kovalevskaia had become attracted to mathematics even as a child, when she was instructed by tutors. To counter the opposition to women as students in the higher Russian academic institutions, she managed by a rather frequently used subterfuge, called fictitious marriage — legal but not sexual — to get out of the country in order to study mathematics. Most of the book is devoted to her development, to the lives of her immediate family and friends, and to her troublesome relations with her husband, who was a geologist. Later, she and her "fictitious" husband did become sexually involved; the marriage produced a daughter.

Kovalevskaia's pursuit of mathematics was her main drive in life. After leaving Russia, she became a student at the University of Heidelberg where she spent several years. There she learned of the work of Karl Theodore Weierstrass (1815–1897), one of the leading mathematicians of Europe and a professor at the University of Berlin. Though she was denied admission to the university, Weierstrass was greatly impressed with her ability and took her on as a private student. With his backing she received a doctorate in mathematics.

Fortunately, Gösta Mittag-Leffler, a liberal Swedish mathematician, then sponsored her for a professorship at the University of Stockholm, a post she took on leaving Berlin and held for many years. During these years, and even earlier, she came to know the leading mathematicians of Europe; it was through recognition in this way, and despite considerable opposition from the establishment, that she was elected to the Russian Imperial Academy of Sciences.

To those who are primarily concerned with the history of mathematics as such, this book will be a little disappointing. There is little mention of Kovalevskaia's actual work, beyond a list of her publications. But it is clear that whereas some mathematicians make brilliant creations as youths, Kovalevskaia developed slowly. In her case the educational difficulties she faced were at least partly responsible, and had she lived longer she would have surely produced much more. Equally interesting to historians of the intellectual connections in the nineteenth century are many of the remarks and details concerning the mathematicians with whom Kovalevskaia kept contact. In particular, her relationship with Weierstrass, who practically adopted her as a daughter, reveals much of that man's noble character.

Ann Hibner Koblitz quite clearly has done a great deal of research in Russia and Stockholm to write this lucid biography. The bibliography is immense and will be of great value to other scholars. Her book should also be of interest to a broad audience, as an example of what a woman could accomplish in nineteenth-century anti-feminist Russia and for the insight into the political and social climate of that period. My only quibble is the word "revolutionary" in the title. Kovalevskaia was indeed what we nowadays know as a feminist. But she was not a militant. □

Morris Kline is Professor Emeritus at the Courant Institute of Mathematical Sciences, New York University. He is author of Mathematical Thought from Ancient to Modern Times *(Oxford University Press, 1972).*



Science and Civilization in China Vol. VI Part II *Agriculture* by Francesca Bray has recently been published by Cambridge University Press and will be reviewed in a forthcoming issue of *Nature.*

First steps in spectroscopy

K. A. McLauchlan

Interpretation of NMR Spectra: An Introductory Audiovisual Programme. By J.D. Coyle, E.J. Haws, K. Miller and K. Norton.
Wiley: 1984. £199.

WILEY's new audiovisual programme comes in a striking package, a large boxed folder which contains four tape cassettes and four boxes (in which are a total of 164 coloured slides), and also 20 booklets. Each tape deals with a particular subject — the chemical shifts of protons, the spin–spin coupling of protons, use of proton spectra for structural assignment and all three of these topics applied to ^{13}C spectra — and is complemented by the slides. Run freely each tape lasts for about 40 minutes, although the package is designed to be interactive with the student so that he may stop and start the tape at will so as to suit his own pace of learning. This is best accomplished with a dedicated slide-tape machine, but an ordinary tape recorder and a slide projector can be used if necessary.

Two types of booklet are supplied, ten of each, together with a correlation chart of proton chemical shifts and a listing of proton coupling constants. The latter is inadequate, even at this level, as is the correlation chart for ^{13}C shifts which is found inconveniently on a slide. One booklet is a very simplistic introduction to NMR spectroscopy. The other is a workbook which has well-defined learning objectives, and in which the student is encouraged to write his replies to answers posed on the tapes and the slides. Answers are supplied on the succeeding slides.

The course has been put together with a great deal of care; the presentation on the tapes is clear and slides are beautifully presented. The coordination of the material between booklet, slide and tape is excellent, too — in theory; my programme did not run properly but that could a quirk of the review package. The tape cues in the operation of the slide–tape machine and the mechanism changes the slide one is looking at to correspond to what is being said, while the tape stops at pre-ordained places to allow the student to think before he goes on.

The material itself is extremely elementary, aimed primarily at trainee technicians but also perhaps at students at the school–university interface or at first-year undergraduates in organic chemistry. In its determination to be straightforward — it is after all an introductory course — it contains slight inexactitudes and half-truths which might worry the better student. Worse, however, is that the package represents the state of knowledge of the subject as it was about 20 years ago. At this level it forms a very basic introduction to the subject which might well build confidence in the student, but if one is going further the background needed would have to be more thorough — Fourier transformation, double resonance, 2D spectroscopy and the various pulse sequences all lie beyond the scope of the Wiley course.

It is initially attractive to think of replacing myself in all those tutorials each week with a magic box, but what are its disadvantages? The publisher extols the virtue of being able to hear the commentary and see the slides at the same time, rather than have your eye skip rapidly from text to diagrams as in a book — fine, provided everyone has a slide-tape machine in his own room! More importantly, this form of teaching seems to be based upon the precept that a student learns in the classroom, but one of the aims of education should be to free him from this restriction. A student should be inquisitive, but how is this virtue encouraged by sitting before a machine which cannot answer questions and which has been programmed by someone else's narrow concept of what should be taught?

Nuclear magnetic resonance is, at best, a subtle, exciting but challenging subject which repays real effort in learning. I am afraid that there is no substitute for a live tutor willing and able to discuss its depths and its applications, to maintain this interest and sense of involvement with developing science on many frontiers. For myself, the Wiley package offers no respite from tutorials, but teachers of the subject at technical colleges may well find it a helpful adjunct to their lectures. □

K.A. McLauchlan is a Lecturer in the Physical Chemistry Laboratory, University of Oxford.

Tigers and calotypes

Brian Coe

Beyond Vision. By Jon Darius.
Oxford University Press: 1984. Pp.224. £15.

IN RECENT years there has been something of a glut of books of historic photographs. As a rule, either the photographs have been chosen to illustrate the history and development of the photographic process, or, based upon the dubious proposition that photography is Art, pictures have been selected for their supposed aesthetic merits. Very few photographs have been presented for their scientific importance — yet, as Dr Darius makes clear in his introductory essay, from its inception photography has been employed to great purpose by scientists. The collection of 100 historic scientific photographs, well reproduced in *Beyond Vision*, is therefore all the more welcome.

Dr Darius has applied very rigid criteria in selecting the photographs, the most important being that each should be unique and should show only what cannot be seen by the unaided human eye. Since the book is presumably intended for a lay readership, it might be argued that slightly less rigid demarcations might have provided greater variety; well over 60 per cent of the illustrations are of astronomical subjects or specialized aspects of physics. In any case, some of the subjects shown appear to fall outside the limits set. The record of the Tasmanian tiger, now extinct, is still no more than a fascinating document of a subject visible to the unaided eye. And the rather pretty colour photograph of the H-bomb test at Eniwetok is no more than a plain record; why were the extraordinary sequence photographs of the first microseconds of the fireball made by Dr Edgerton's highspeed camera not chosen?

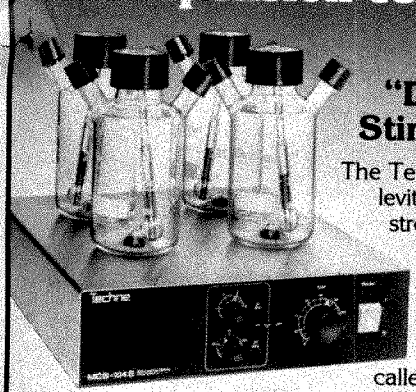
The inclusion of the contentious Skaife "instantaneous" photograph of a mortar shell in flight is also a little odd. The picture has clearly been retouched, and even if it is authentic its purpose was not scientific but promotional. There are questionable omissions, too. For instance, no example of the forensic use of ultra-violet and infra-red photography is included, and there is not a single scanning electron micrograph of any sort, despite the exceedingly wide range of applications in science and industry. To be fair, in reducing the entire output of 140 years of scientific photography to 100 examples, the author could not expect to please everyone.

The introductory essay is lucid and informative but is marred by several errors. The skeleton outline of photographic processes is misleading; it makes no mention of the key fact that the calotype process made paper negatives which could be printed. To use the description "coated with emulsion" to cover such diverse processes as the Daguerreotype (a silver surface sensitized by iodine vapour), the calotype (paper impregnated with silver salts), the collodion process (a glass plate coated with halide-containing collodion and sensitized in silver nitrate) and the dryplate process (a suspension of silver salts in melted gelatin coated onto glass or paper) is carrying simplification too far. Talbot's early photomicrographs, *c.* 1837, were not calotypes, but were made by his earlier "photogenic drawing" process.

Seen against Dr Darius's achievement in bringing these photographs together, these are minor criticisms. Overall, the book is a most valuable contribution to the literature on the history of photography. We must hope that it will be sufficiently successful to be followed by a second selection revealing more of the wonders of scientific photography. □

Brian Coe is Curator of the Kodak Museum at Harrow, Middlesex.

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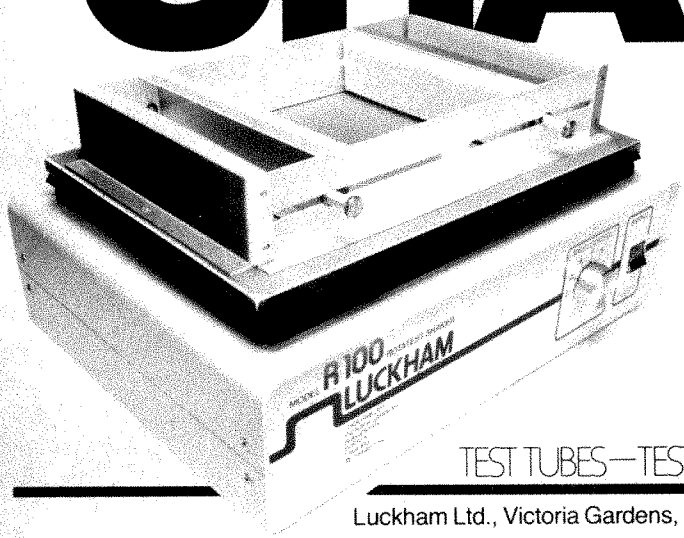
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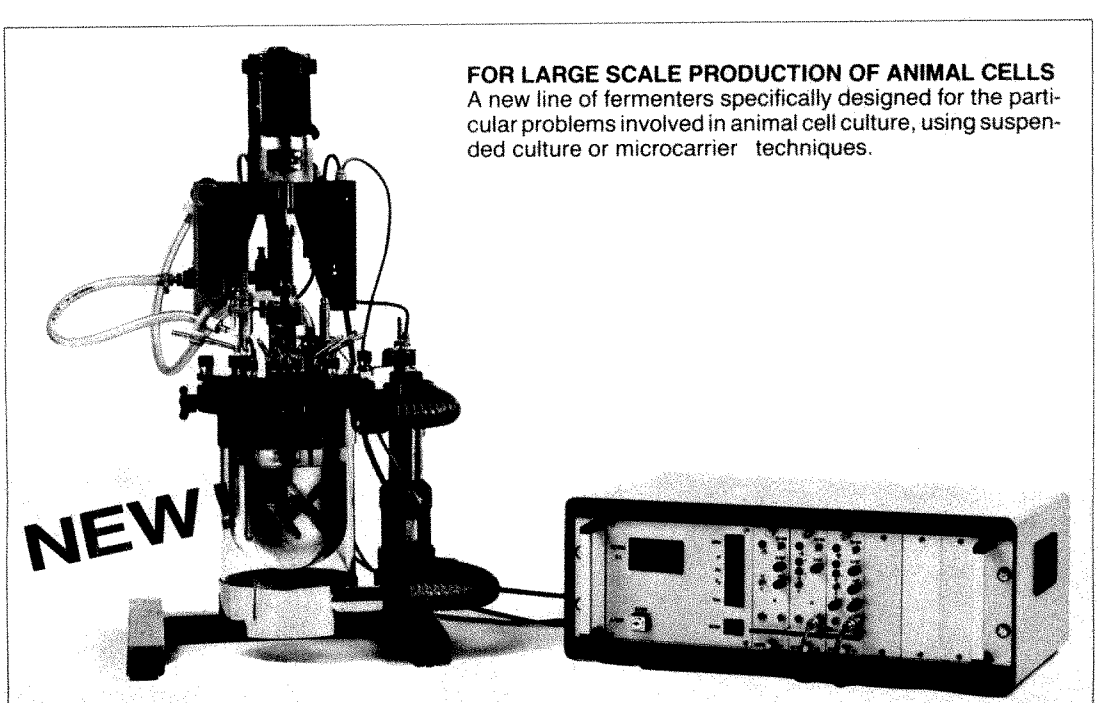
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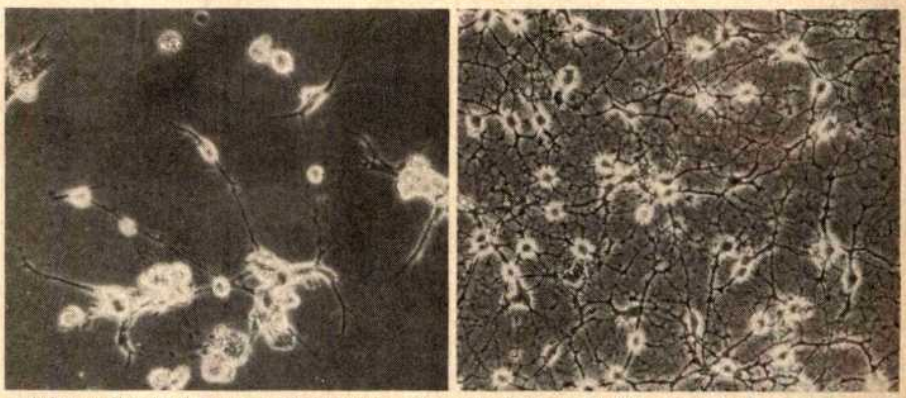
New for tissue culture

The eleven items here cover recently announced products of use in the tissue culture laboratory

● A new range of culture dishes is now available from International Bio-Technologies (IBT) Ltd. The dishes are coated with a naturally produced extracellular matrix (ECM), a basement membrane-like substratum which promotes cell attachment, proliferation and differentiation. The ECM is secreted by endothelial cells cultured on conventional tissue culture plastic. The cells are then removed, leaving the underlying ECM intact and firmly attached to the surface of the dish. The composition and supramolecular arrangement of the ECM is similar to basement membranes occurring *in vivo*. Cells placed in contact with ECM attach rapidly, exhibit high plating and cloning efficiences, proliferate rapidly, reach a h saturation density and have much ver requirements for serum and added growth factors than cells in conventional dishes. They also respond better to physiologically occurring hormones and express differentiated functions. These effects cannot be obtained by cell plating on isolated fibronectin, laminin or each of the collagen types and on reconstituted matrices. Among the cell types showing a favourable response to ECM are normal and carcinomatous human epithelial cells derived from surgical biopsies, human amniotic fluid cells, bovine granulosa, adrenal cortex and endothelial cells, rat pituitary, oligodendrocytes and dorsal root ganglia cells. The coating is available on any size of tissue culure dish, T-flasks, multiwell plates and coverslips.
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● The culture of hybridomas and diploid human cells has become increasingly important and this has led LSL Biolafitte of Princeton, New Jersey, to design a range of vessels specifically suited to this purpose. LSL Biolafitte offers eight vessels with working volumes from one litre to 330 litres. Each vessel has optimized geometry with a working height to diameter ratio of 1:1 and hemispherically shaped base. An effective low speed, low shear agitation system is incorporated into each vessel to gently agitate the cells. Harvesting is achieved by a conveniently located valve which has two positions allowing total drainage or liquid removal only. Other system design features are included, or are available as optional accessories, to ensure strict containment in processes where this is an important factor.
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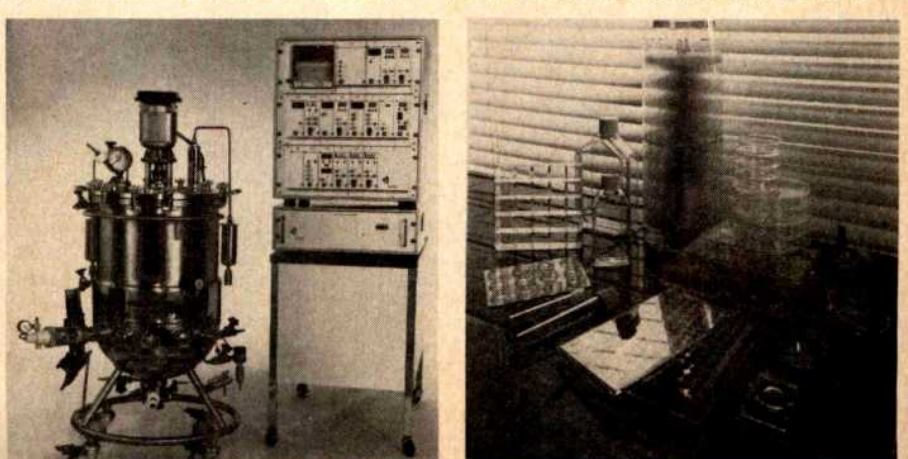


Adult rat oligodendrocytes maintained on conventional plastic (left) and on plastic coated with IBT's extracellular matrix preparation.

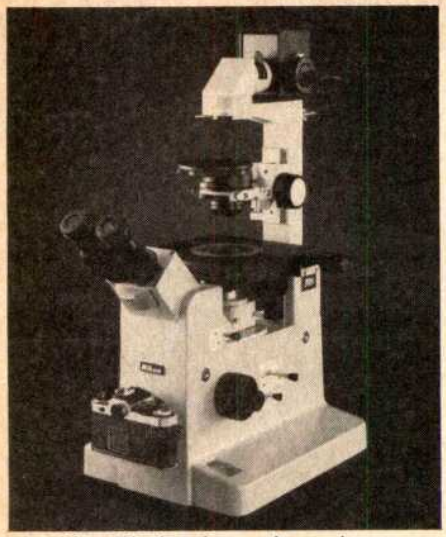
● The Sterilin range of tissue culture plastics includes dishes, tubes, flasks, plates and bulk vessels designed to ensure efficient attachment and growth of cell monolayers. All equipment is manufactured from non-toxic polystyrene, providing a high standard of finish and good optical properties for microscopy. Every batch is tested for sterility and the ability to support cell growth. Petri dishes are available in a wide range of sizes and types — the 50mm and 60mm dishes can be supplied with single or triple vents and a multi-well dish is also available. Sterilin test tubes come in two sizes: the 13ml tube is fitted with a leak-proof screw cap, and the 5ml tube has a two-position cap for gas exchange. For research and clinical studies involving human and animal cells, two sizes of tissue culture flask are available. Graduated in mls, the wide necks (canted in the 25cm^2 size) allow easy pipette access and non-toxic leakproof screw caps are fitted for safety. A stacking rim is provided for extra stability. All flasks are individually pressure-tested to eliminate the possibility of leakage through the welded seams.
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● Sera-Lab fetal calf sera are now available in graded batches to select those which will support the rat and mouse myelomas used to make cell hybrids. Each batch of serum is routinely tested for the ability to support the growth of 11 cell lines, so at no extra cost the customer can request a serum to suit a particular need.
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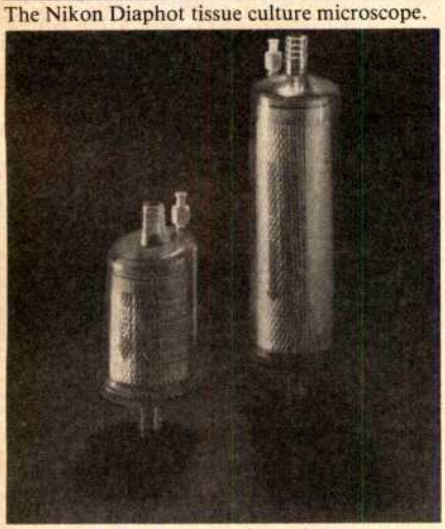
● A kit for preparing serum-free media has been announced by Gibco. The Select-a-Factor kit contains 1 ml sample sizes of the more commonly-required factors and technical information on how to grow cells in a serum-free environment. The technical information describes four experiments that explain the preparation of serum-free media. The kit includes 1 litre of powdered medium (Dulbecco's minimum essential medium: nutrient mixture F-12 with 15-mM HEPES), trypsin inhibitor and 14 ready-to-use factors (EGF, FGF, fibronectin, poly-D-lysine, vitamins C and E, insulin, transferrin, hydrocortisone, progesterone, selenium, putrescine, trace element mix, and T_3). The factors are also available individually for the preparation of subsequent media.
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One of the LSL Biolafitte culture vessels (left) and the Sterilin range of tissue culture plastics.



The Nikon Diaphot tissue culture microscope.



Disposable filter capsules from Gelman.

● The range of accessories available for the Nikon-Diaphot inverted tissue culture microscope now includes systems for time-lapse cine photomicrography or closed circuit television that can be fitted without disturbing the camera mounted on the front. Motor driven computerized stepping stages can be fitted to the Diaphot for image analysis work, and for environmental control, the Nikon constant temperature incubator can provide warm air at 37°C. An override allows variation in temperature of plus or minus 4°C.
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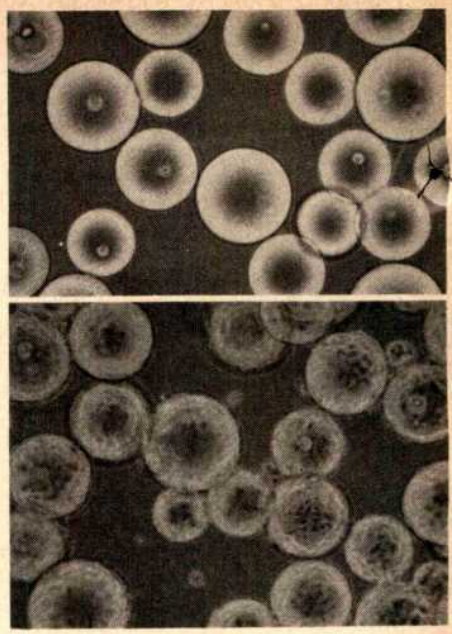
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● Gelibead, a gelatin microcarrier developed by KC Biological, solves two of the major problems associated with microcarrier culture. First, enzymatic removal of viable cells from the microcarrier is possible and second, eluted cells can be readily removed from the spent microcarriers. Gelibead microcarriers permit highly efficient cell harvesting because they completely dissolve in a mixture of trypsin, dispase and EDTA. Exposing Gelibeads for about 20 minutes to this mixture results in complete dissolution of themicrocarriers with very high cell recoveries. Gelibeads can be attached to a wide range of cell types, provides high density culture (about 3,800 cm^2 per gram dry weight) and gives high cell yields.
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● In the new Luckham RMV tissue culture roller apparatus the rollers rotate at a set speed with the minimum of variation, resulting in steady even culture growth. All rollers are driven, they are covered in rubber and support the bottle along its whole length. The unit is available in two speed ranges, 2–10.5 revolutions per hour or 40–180 per hour (other speed ranges are available to special order). There is an analogue speed meter so that experimental conditions can be precisely reproduced. Luckham has also just introduced the TC 1000, a drum roller designed for the rotation of 16-mm diameter tubes. The unit has a variable speed between 0.5 and 60 r.p.m. Speed is electronically controlled, and read out via an analogue meter. Luckham manufactures a range of racks designed for tissue culture work, with a variable angle between 0 and 5 degrees.
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● Gelman's new "Culture Capsule" and "Maxi Culture Capsule" are disposable, ready-to-use capsule filtration devices for sterilizing large volumes of tissue culture media. These presterilized capsules incorporate two layers of 0.2 μm low-protein-binding HT Tuffryn membrane, and are designed and certified to assure their integrity for tissue culture media sterilization. Designed for use with positive pressure filtration, the Culture Capsule and Maxi Culture Capsule are suitable for in-line applications using a pressure vessel or peristaltic pump. For sterilization of culture media containing serum, either capsule may be used as the final sterilizing unit in conjunction with prefilters. Disposable filters minimize set-up time and risk of cross-contamination. The Culture Capsule (product no. 12140) provides 500 cm^2 typical filter area, suitable for sterilization of up to 10 litres of solution. The Maxi Culture Capsule (no. 12141) offers 1,290 cm^2 typical filter area, and is ideal for larger volume applications of up to 20 litres. Both capsules are available from the following laboratory supply dealers: American Scientific Products, Fisher Scientific, VWR Scientific and Curtin Matheson Scientific.
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The Gelibead microcarrier without cells (top) and with attached MDCK cells.

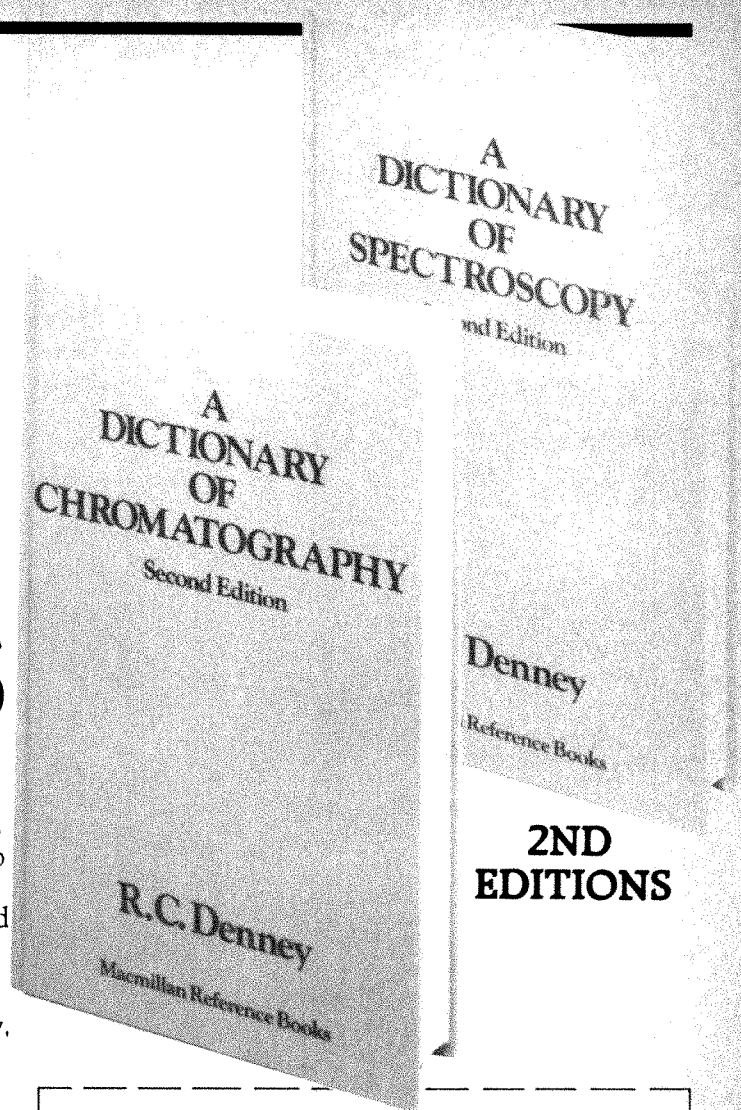


Luckham's new tissue culture roller.

● A new product from Hana Biologics is intended to reduce the requirement for serum in mammalian cell culture. SerXtend is a combination of growth factors which when added to fetal bovine or other sera will allow for a 50% or greater reduction in the concentration of serum used in cell culture. It is a non-serum based product, formulated and certified by biological activity to allow for lot-to-lot consistency.
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● The new Microtec Swift tissue culture microscope is an inverted microscope which can be fitted with bright field or phase contrast objectives designed to work through the chambers used in tissue culture. Illumination is provided by a 20 watt halogen source and, with the standard condenser, chambers up to 45mm thick can be used. An extra long working distance condenser system, with a working distance of 60mm, is available for use with thicker chambers. A camera port is a standard feature enabling a 35mm camera to be plugged into the side of the microscope. The coaxial coarse and fine focusing mechanism operates entirely on the objectives, so that viewing head, camera and specimen stage are in a fixed position.
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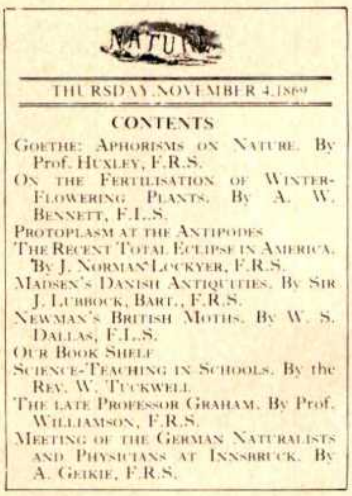
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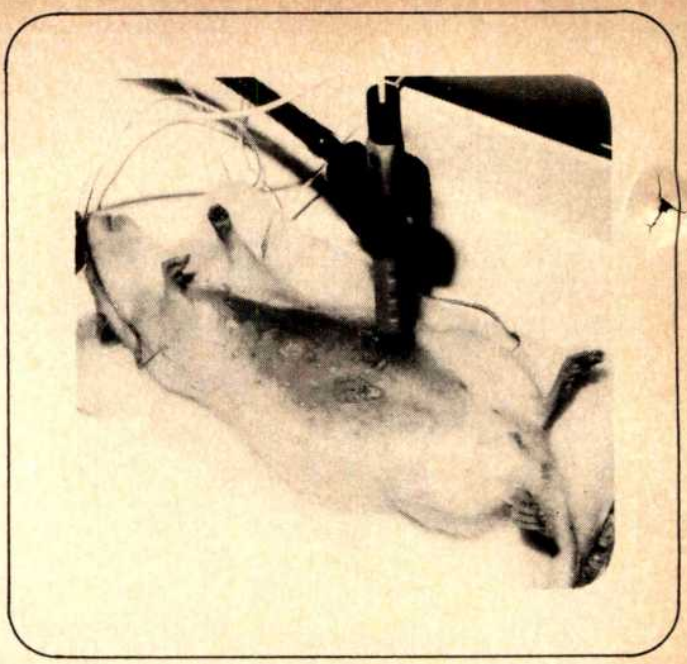
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UNIVERSITY OF LIVERPOOL

DEPARTMENT OF SURGERY

TECHNICIAN GRADE 7

to organise the provision of a technical service in research laboratories which undertake a wide variety of biochemical, histological and immunological work. Other duties include assistance with organising examinations and responsibility for budgeting research grants.

Candidates must possess HNC or appropriate equivalent as minimum qualification plus ten years relevant experience including staff supervision.

Salary within range £8,385-£9,418 per annum (under review).

Applications forms may be obtained from The Registrar, The University, PO Box 147, Liverpool, L69 3BX. Quote ref RV/519N.

(2075)A

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF ANATOMY AND EMBRYOLOGY

LECTURESHIP

Applications invited for a LECTURESHIP. No particular field is sought, but applicants are expected to have a strong commitment to original research. The Department has broad interests and excellent facilities. The position offered is in lieu of a Wellcome Trust Senior Lectureship, so "the tenure of the award (post) is for no less than an initial five years, followed by possibly a further one or more periods each of three years".

Applications, together with full cv (including publications list) and the names of three referees, should be sent to: Assistant Secretary (Personnel), University College London, Gower Street, London WC1E 6BT. (2128)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PHYSICS

PHYSICIST/CHEMIST

Applications are invited for a pos doctoral Physicist or Chemist t work for 18 months in the firs instance on the electronic propertie of conducting polymers and organi semiconductors. The project covers wide range of physical measure ments, including optical and tran port properties, and will involve th preparation, characterisation an chemical or electrochemical dopin of these materials.

Salary in the range £6,745 to £9,42 pa.

Applications to Mr J Deakin Secretary of the Department Physics, Cavendish Laborator Madingley Road, Cambridge CB 0HE, quoting ref RF. Closing date June. (2078)A

Clinical Research Associate

Greenford, Middx.

Glaxo Laboratories Limited is a leading British pharmaceutical company responsible for the promotion of products of original research developed and manufactured in the UK. We are now seeking to recruit an experienced Clinical Research Associate to join the Company's Medical Services Department.

You will be required to arrange Clinical Trials and bring them to a satisfactory conclusion by ensuring that data is collected and handled efficiently once received. You will be involved in the preparation of protocols and the administration connected with the study and you will be expected to meet Clinical Trialists on a regular and frequent basis.

In addition to a degree in pharmacy or a suitable science, you should have experience in the field of gastroenterology, possess a dynamic and friendly personality and be able to communicate effectively, both verbally and in writing.

The attractive conditions of employment include non-contributory pension scheme, four weeks' and two days' holiday, a subsidised cafeteria, a Group Bonus Scheme and excellent sports and social facilities.

Please telephone for an application form to: Mr. M. E. Bates, Senior Personnel Officer, Glaxo Laboratories Ltd., Horsenden House, Oldfield Lane North, Greenford, Middx. on 01-422 3434, ext. 2837 quoting ref. MEB/736.

(2130)A

Glaxo Laboratories Ltd

Molecular Biologists

A challenging career opportunity in genetic engineering.

ICI Australia Operations Pty. Ltd. Central Research Laboratories in Melbourne, are equipped with a comprehensive range of biological and chemical research facilities. The laboratories have a staff of 160, including more than 100 graduates. The Company has been a leader in research and development in Australia for over 20 years and has strong contacts with other research institutions throughout Australia, including those involved in biotechnology.

We are seeking two Molecular Biologists to participate in the establishment of a strong new team in the field of genetic engineering. The immediate aim of the team is the expression of eukaryote genes in bacteria and there will be a long-term commitment to the genetic manipulation of micro-organisms to meet a range of company targets.

Consideration will be given to applicants either completing a Ph.D. in recombinant DNA work or with post-doctoral experience in a laboratory of international standing in the same field.

The salary offered initially will depend on qualifications and experience and is subject to annual review. Benefits include contributory superannuation scheme and supplementary non-contributory medical benefits scheme.

ICI is an equal opportunity employer.

Personal details and a full account of research experience, techniques and publications with the names of two or more referees, should be sent to:

Personnel Manager,

ICI Australia Limited

G.P.O. Box 4311,
Melbourne, Vic. 3001, Australia.

The ICI Roundel is a Trademark (W1006)A

CSIRO

RESEARCH SCIENTIST/ SENIOR RESEARCH SCIENTIST

A$25,342 – A$37,274

Division of Entomology, Canberra ACT

Field: Application of Molecular Biology in Entomology.

General: CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Entomology is a member of the Institute of Biological Resources.

The Division of Entomology conducts research on entomological problems of both basic and applied importance. It recently initiated a molecular program aimed at investigating how genetic information is stored in the insect genome and used during development. Such knowledge should assist in the identification of novel methods of pest control. The Division has a well equipped molecular biology laboratory and maintains active research programs in insect biochemistry, physiology, behaviour and genetics. The Australian sheep blowfly *Lucilia cuprina* and *Drosophila melanogaster* are major experimental organisms used in these programs.

Duties: The successful applicant will combine a knowledge of genetics and molecular biology to elucidate the molecular mechanisms determined by genes responsible for insect development and pattern formation. An interaction is envisaged with other staff currently using recombinant DNA techniques and insect transformation to study chromosome structure and gene function.

Qualifications: Applicants should have a Ph.D. or equivalent in a relevant field, preferably with research experience in insect biochemistry, physiology and genetics, and must be competent in analyses of DNA and RNA, molecular cloning and nucleic acid sequencing.

Tenure: Indefinite. Australian Government superannuation benefits available.

Applications: Stating full personal and professional details, the names of at least two referees, and quoting reference no A0605 should be forwarded to:

The Chief
CSIRO Division of Entomology
CANBERRA CITY ACT 2601
AUSTRALIA
by June 22, 1984. (W996)A

CHAIR IN ORGANIC CHEMISTRY

Applications are invited, from candidates of either sex, for appointment to a Chair in Organic Chemistry in the Department of Chemistry.

The person appointed will be ready to sustain and develop the distinctive industrial interests of the Department. His/her research activities may be in any branch of Organic Chemistry, but preference will be given to candidates with an interest in synthetic organic chemistry in its widest context. A good record of, and potential for, attracting industrial support for the research will be a distinct advantage.

Salary will be in the Professorial range, minimum currently £17,275 p.a., but under review.

Informal enquiries may be made of Professor J O Williams (Chemistry Department) or of The Registrar.

Requests for application forms and further particulars, quoting reference CH/70/A1, should be addressed to The Registrar, Room B9 UMIST, PO Box 88, Manchester M60 1QD. Completed applications should be returned as soon as possible.

(2104)A

UMIST

The University of Manchester Institute of Science and Technology

Head of Pharmacology

KENT

The Wellcome Foundation Limited is a major group of pharmaceutical and chemical companies with headquarters in the UK and a turnover close to £700m.

This is a key senior appointment reporting to the Director of Therapeutic Research Division. The department is active on a wide variety of forward-looking research projects and comprises some 60 personnel – including scientists, technicians and clerical/secretarial staff.

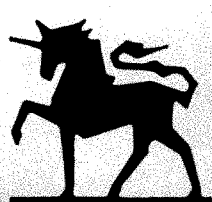
The prime responsibility of the position will be to direct, manage and co-ordinate the department's activities and resources to meet the overall research objectives within defined cost, quality and time parameters. There is also a requirement to co-ordinate "the development of the science" and ensure that a consistency of high quality of scientific and technical performance is sustained.

Candidates must be able to demonstrate a substantial scientific and managerial track record in a relevant field and have the maturity to promote and develop scientific excellence through personal leadership and motivation. As a senior scientist, the successful candidate must also be capable of contributing to the general debate on scientific research methodology objectives and maintaining a contemporary scientific perspective in this important area of research.

The salary and benefits package will reflect the importance of the position and includes a company car and generous assistance with relocation expenses.

Please write with full c.v. to
Alan Murdoch, Personnel Officer,
The Wellcome Research Laboratories,
Langley Court, Beckenham, Kent BR3 3BS, England.

(2108)A



Wellcome

IRI

Inveresk Research International

MAJOR OPPORTUNITIES IN BIOTECHNOLOGY

Location — Scotland

Inveresk Research International Limited has substantial development in Biotechnology underway and is already a leading force in the contract research area. Activities focus on monoclonal antibodies for diagnosis and therapy and in genetic engineering. Our laboratories are modern and well equipped and we are now moving into a phase of further expansion as a result of which the following vacancies have now become available:-

HEAD OF IMMUNOLOGY

Candidates for this appointment should have proven management capabilities as the person appointed will be responsible for our human monoclonal antibody research, which will involve running an already experienced team. The desired qualifications are a PhD with at least several years' industrial experience.

At least initially, this post will be located in our Glasgow laboratories in George Street. (Reference Number: 3502).

CONTRACT BIOTECHNOLOGY MARKETING MANAGER

Candidates for this appointment must have an ability to broaden our client base in the Biotechnology area. An aptitude in proposal writing and/or knowledge of licensing of intellectual property would be added advantages. The required academic qualifications are at least an Honours Degree.

This post will be based at our Biotechnology laboratories in Musselburgh. (Reference Number: 2590).

Applicants for either of the above posts must have practical experience in appropriate fields and must have the necessary drive and initiative to operate successfully in a commercial environment. Salary in both cases will be according to qualifications and relevant experience.

In addition, the Company offers modern staff benefit schemes, including free BUPA membership, and a modern working environment. Relocation expenses may also be provided, where appropriate.

A full c.v., should be submitted in confidence to the Personnel Department, Inveresk Research International Limited, Inveresk Gate, Musselburgh EH21 7UB, quoting the appropriate reference number. (2107)A

UNIVERSITY OF LEICESTER
Leicester Biocentre

POSTDOCTORAL RESEARCH ASSOCIATE
Yeast Molecular Biology

Applications are invited for a postdoctoral research position, funded by a major UK Food and Drink company, to undertake research aimed at the improvement of existing yeast strains for commercial applications.

The project will involve the use of genetical, biochemical and recombinant DNA methods and Applicants should hold a PhD in a relevant subject. The contract is for a period of 3 years and appointment will be made within the Grade 1A salary scale (ie £7,190 — £11,615).

Applications, with full curriculum vitae should be sent to the Secretary, Leicester Biocentre, University of Leicester, Medical Sciences Building, Leicester LE1 7RH. (2105)A

VIRGINIA COMMONWEALTH UNIVERSITY
MEDICAL COLLEGE OF VIRGINIA

DEPARTMENT OF HUMAN GENETICS

HUMAN MOLECULAR GENETICIST

The Department of Human Genetics of the Medical College of Virginia is seeking applications for an assistant professor position. Candidates must have a doctoral degree, at least two years postdoctoral experience and a strong background in Human Genetics. The successful applicant will be expected to establish an independent research program and to participate in graduate studies.

To apply, send vitae and three letters of reference to Dr W E Nance, Box 33, MCV Station, Richmond, VA 23298.

Equal Opportunity/Affirmative Action Employer. (NW539)A

THE UNIVERSITY OF WESTERN AUSTRALIA
Perth

CHAIR IN MICROBIOLOGY

Applications are invited for appointment to the Chair in Microbiology which became vacant at the end of 1983 on the retirement of Professor N F Stanley. A newly established Chair in Clinical Microbiology is currently under advertisement.

The appointee to this Chair will be expected to provide a high level of academic leadership and to play a major role in teaching and research and in the administration of the Department of Microbiology. The appointee will also be expected to be from time to time Head of Department. The Department is located in a modern, well equipped building at the Queen Elizabeth II Medical Centre and is responsible for the teaching of Microbiology to science, medical and dental students. The research sections of the Department comprise functional units in bacteriology and genetics, virology and epidemiology, immunology, environmental microbiology and clinical microbiology.

Candidates should have an outstanding record of research in microbiology. Although applications will be welcome from scholars in any are of microbiology, preference may be given to candidates with a strong background in virology and research interests in molecular aspects of the discipline, or in molecular biology with research interests in recombinant DNA technology.

The current salary for a Professor is $A51006 per annum, plus a clinical loading of $A4566 per annum for a medically qualified candidate. Benefits include superannuation, fares to Perth for appointee and dependent family, removal allowance, study leave and long service leave and eligibility for interstate travel grants and housing loan scheme.

Prospective applicants should write to the Staffing Officer, the University of Western Australia, or to the Secretary General, Association of Commonwealth Universities, (Appts), 36 Gordon Square, London WC1H 0PF, for further particulars before submitting applications.

Applications in duplicate stating full personal particulars, qualifications, experience and the names and addresses of three referees should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia, 6009 by 27 July 1984. (W1012)

POSTDOCTORAL POSITION

available for individual to carry out in vitro mutagenesis of cloned, selectable mammalian genes and to characterize their expression after transfection into cultured cells. Molecular genetic experience desirable. Send CV and names of 3 references to Dr Peter J Stambrook, Department of Anatomy and Cell Biology, University of Cincinnati Medical Center, Cincinnati, OH 45267-0521. (NW540)A

THE UNIVERSITY OF WESTERN AUSTRALIA
Perth

CHAIR IN CLINICAL MICROBIOLOGY

Applications are invited for appointment to the newly established Chair in Clinical Microbiology. This position is one of two Chairs in the Department of Microbiology, the other being also vacant with the retirement of Professor N F Stanley.

The Department of Microbiology is located in a modern, well equipped building at the Queen Elizabeth II Medical Centre, which includes the Medical School, Sir Charles Gairdner Hospital and State Health Laboratory Services. The appointee will be expected to play a major role in teaching and research and in the administration of the Department, to undertake and co-ordinate medical student teaching and postgraduate training in microbiology and to provide leadership and expert clinical advice on microbiology and infectious diseases to hospital clinical staff. The appointee will also be expected to direct and administer the Combined Clinical Microbiology Laboratory Service (which provides routine microbiology services to Sir Charles Gairdner Hospital and other public hospitals in Western Australia) and the associated specialist laboratories concerned with clinical and public health work in a combined microbiology service to hospitals and the public health authority.

The Department of Microbiology is responsible for teaching medical, dental and science students. The research sections of the Department comprise functional units in bacteriology and genetics, virology and epidemiology, immunology, environmental microbiology and clinical microbiology.

Candidates should hold a medical degree registerable in Western Australia, together with postgraduate qualifications appropriate for appointment at senior consultancy status. Candidates must also have extensive experience in clinical microbiology and knowledge of and expertise in diagnostic laboratory procedures. Beds will be available for a suitable appointee.

The current salary for a Professor is $A51006 per annum, plus a clinical loading of $A9120 per annum. Benefits include superannuation, fares to Perth for appointee and dependent family, removal allowance, study leave and long service leave and eligibility for interstate travel grants and housing loan scheme. Limited rights of private practice available.

Prospective applicants should write to the Staffing Officer, the University of Western Australia, or to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, for further particulars before submitting applications.

Applications, in duplicate, stating full personal particulars, qualifications, experience and the names and addresses of three referees should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia, 6009, by 27 July 1984. (W1011)A

University College of Swansea

Wellcome Lectureship in Magnetism Applied to Medicine

Applications are invited from candidates under 35, who are not holders of a tenured University post, for a Wellcome Lectureship in the Department of Physics. The Lecturer will be expected to work closely with the Swansea In vivo Analysis Research Group (SIVARG) which is a collaborative group involving the Department of Medical Physics at Singleton Hospital and Medical Consultants of the West Glamorgan Health Authority carrying out research on in vivo body element analysis.

The appointment, which will date from October 1, 1984, will be for three years in the first instance with the possibility of renewal for a further two years, subject to satisfactory review. The salary will be on the scale £7190-£14125 per annum, the commencing salary depending upon age.

Informal enquiries may be made to Professor J. Dutton, Department of Physics, but further particulars and application forms (2 copies) must be obtained from the Personnel Office, University College of Swansea, Singleton Park, Swansea, SA2 8PP, to which office they should be returned by June 13, 1984.

(2109)A

Monsanto, a large organization with European Headquarters in Brussels and research facilities in pleasant surroundings at Louvain-La-Neuve, 30 km south of Brussels, is expanding its agriculture research programs. The Biological Evaluation section intends to make new appointments for (m/f)

Scientists

with the ability to carry out and direct applied research projects. These projects will be related to the discovery and development of plant growth regulators and fungicides for use in major arable crops.

Applicants should have a PhD in agronomy, plant pathology or related plant sciences. A strong background in plant/crop physiology, and some experience of field experimentation, would be advantageous.

Candidates should apply, enclosing a full curriculum vitae, to Personnel Department, Monsanto Technical Center, rue Laid Burniat, B-1348 Louvain-La-Neuve, Belgium.

Monsanto

(W995)A

TRUDEAU INSTITUTE

A Postdoctoral research position in infectious disease

is currently available to study interactions between several intracellular microbial pathogens and the cellular defenses in both normal and immunosuppressed animals. Current research is directed towards understanding cellular interactions responsible for the modulation of delayed-type hypersensitivity and acquired antimicrobial resistance during the development of chronic systemic disease in inbred mice. Candidates should have a strong background in infection-immunity or in immunology.

The Trudeau Institute is a private, not-for-profit basic medical research institution situated on the shores of Lower Saranac Lake, about a mile from the village of Saranac Lake and 10 miles from Lake Placid, NY. On-campus housing is available. Salary is at appropriate NIH levels.

Send curriculum vitae and names of three references to: Dr Frank M. Collins, Trudeau Institute, P.O. Box 59 Saranac Lake, N.Y. 12983. An Equal Opportunity Employer.

(NW525)A

The Queen's University of Belfast

GRADUATE/ POST-DOCTORAL RESEARCH ASSISTANT Molecular Biology

Department of Biochemistry

This post, supported by the Multiple Sclerosis Society, is tenable in the Biochemistry Department from 1st October, 1984 for one year. The appointee should preferably have interests in molecular virology and some experience in recombinant DNA methods as applied to the study of multiple sclerosis. Candidates should preferably hold or expect to receive a good honours degree in a relevant discipline. A higher degree with research experience in recombinant DNA methodology while not essential would be an advantage.

The appointee will be made within the salary range: £6,312-£8,532 per annum, placing depending on qualifications and age.

Applicants should send a full curriculum vitae including the names and addresses of two referees to the Personnel Officer, The Queen's University of Belfast, Northern Ireland, BT7 1NN. Closing date: 5th June, 1984. (Please quote Ref. 84/N).

(2118)A

Agricultural and Food Research Council FOOD RESEARCH INSTITUTE Process Physics Division Theory of Food Systems

A MATHEMATICIAN OR THEORETICAL PHYSICIST

Higher Scientific Officer

is required to join the Process Physics Division in the Institute. The successful candidate will have a strong interest in the rheology of both Newtonian and non-Newtonian materials and use both analytical and numerical mathematical methods to gain an understanding of their behaviour in both rheometric and non-rheometric situations. A strong commitment to relating the results to experiments is essential.

Qualifications: 1st or upper 2nd class honours degree in Physics or Applied Mathematics and at least two years' relevant post-graduate experience, preferably a PhD, in Theoretical Physics or Applied Mathematics with experience in fluid mechanics, rheology or statistical mechanics.

Salary: On a scale £7,149-£9,561 (under review). Non-contributory superannuation scheme.

The Agricultural and Food Research Council is an Equal Opportunities Employer.

Further particulars and application form from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA, quoting reference no. 84/ NP/ 7.

Closing date: 8th June 1984

(2090)A

GERMAN CANCER RESEARCH CENTER HEIDELBERG

CELL/ MOLECULAR BIOLOGIST

A postdoctoral position (2 years initially is available to join a research group working on the control of gene expression. The research will focus on the tissue specific expression of the chicken lysozyme gene and the rat tyrosine aminotransferase gene and its control by steroid hormones and cAMP. Applicants with experience in molecular and cell biology and/or somatic cell genetics will be preferred.

Applications with full CV, description of scientific experience and the names of two referees should be sent to: Prof. Dr. Günther Schütz, Institute of Cell and Tumor Biology, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, D - 6900 Heidelberg, F.R.G. (W998)A

POSTGRADUATE RESEARCH IN GEOLOGY

(2 POSTS)

Salary £5649 — £6363
Pay award pending

Applications are invited from candidates with, or expecting to obtain a good honours degree in either Geology (for both projects) or joint Geology/Chemistry (for project 2) for the following: —

1. Structure, lithostratigraphy and sedimentology of the Lower Devonian rocks of South Devon.
2. Temporal and spacial variations in the chemistry and mineralogy of historic larvas from Mount Etna, Sicily.

Research Assistants are expected to register for a CNAA higher degree. Appointments are for a period of two years with a possibility of a third year (fixed term contract).

Further details and application forms to be returned by Friday 15th June 1984 can be obtained from the Personnel Office, Plymouth Polytechnic, Drake Circus, Plymouth PL4 8AA. Tel: (0752) 264639 (NB Please specify clearly for which project you are applying). (2081)A

Plymouth Polytechnic

UNIVERSITY OF OXFORD

DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL RESEARCH ASSISTANT GRADE RS1A

(Ref: B/R/106)

A Post-doctoral Research Assistant is required to work with Drs A J and S M Kingsman on the control of gene expression and plasmid replication in Saccharomyces cerevisiae. The post is available for three years. The salary will be on the scale £7,190 — £9,425 according to qualifications and experience and with membership of USS.

Applications, including a full curriculum vitae and names and addresses of two referees, should be sent by 30th June 1984 to The Administrator at the address below.

DEPARTMENTAL DEMONSTRATORSHIP

(Ref: B/D/105)

It is proposed to appoint a Departmental Demonstrator from 1st October, 1984, or as soon as possible thereafter and preference may be given to candidates whose interests are in physical biochemistry. The successful candidate will be expected to take part in departmental teaching and to conduct his or her own research. The appointment is for a period not exceeding three years in the first instance. The salary will be on the scale £6,310 — £8,975 pa according to qualifications and experience and with membership of USS.

Applications (3 copies), including the names and addresses of two referees, should be sent, not later than 30th June, 1984, to The Administrator, Department of Biochemistry, South Parks Road, Oxford OX1 3QU. (2146)A

JOHN INNES INSTITUTE

PLANT MOLECULAR BIOLOGIST & CELL BIOLOGIST

Higher Scientific Officers, Band 1

Applications are invited for two posts to extend the Institute's programme in molecular plant pathology. Both posts are for three years duration.

The appointees will collaborate with other members of the Norwich Molecular Plant Pathology Group (NOMPAG), consisting of members of the John Innes Institute staff and faculty of the School of Biological Sciences, University of East Anglia, who are applying a range of molecular biological techniques to selected problems in plant pathology.

It is envisaged that Post 1 will be filled by a molecular biologist/biochemist who will investigate and isolate plant genes which are expressed following infection by pathogens, while Post 2 requires a cell biologist to investigate (probably by immunochemical procedures plant cell wall structure with particular reference to the possible involvement of wall polymers in host-pathogen recognition reactions. Further details may be obtained from Dr M J Daniels (Post 1) and Dr K Roberts (Post 2).

Applicants should have a good honours degree, with ideally a PhD and post-doctoral training in a relevant area of biology. Salary will be dependent on age and experience, on the Higher Scientific Officer scale in the range of £7149-£9561 (pay award pending). The John Innes Institute is an Equal Opportunities Employer.

In accordance with the provision of the Employment Protection (Consolidation) Act 1978, staff on short term appointments are required to waive any statutory rights to redundancy payments or to make claims of unfair dismissal, in respect of dismissal at the end of the three years project.

Applications giving full particulars and experience together with the names of two referees should be sent to the Secretary, John Innes Institute, Colney Lane, Norwich, Norfolk NR4 7UH, England quoting reference Gen/161 (Post 1) or CB/162 (Post 2) by 16 July 1984. (2093)A

IMPERIAL COLLEGE

DEPARTMENT OF CHEMISTRY

Applications are invited for a

POST-DOCTORAL RESEARCH ASSISTANT

for two years starting from 1 September 1984 to work with Professor W J Albery and Professor B C H Steele on porous oxide electrodes.

Applications including a curriculum vitae and the names of two referees should be sent as soon as possible to Professor W J Albery, Department of Chemistry, Imperial College, London SW7 2AY. (2123)A

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF BIOCHEMISTRY

WELLCOME TRUST TEMPORARY LECTURESHIP

Applications are invited for the po of Temporary Lecturer in the Depar ment of Biochemistry, funded by th Wellcome Trust, tenable from 1 October 1984 for 3 years in the fir instance, with the possibility renewal for a further 2 years. Appl cants should have an interest in mem brane biochemistry. Preference ma be given to applicants with interests i tissue culture and/or monoclon antibody techniques and whos research interests complement thos currently active in the Departmen The successful applicant, who will b under 35 and not hold a tenured pos will be integrated into the unde graduate teaching programme of th Department which is to both scienc and medical students.

Applications (7 copies from appl cants within the U.K. and one fro others) in the form of a curriculu vitae and the names and addresses three referees and a 500 word sum mary of the applicant's proposed re search project should be sent befor 16th June 1984 to Mr A J Smal Staffing Secretary, The University Highfield, Southampton, SO9 5NH Please quote reference No AJS/84/21/N. (2113)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BIOCHEMISTRY

EXPERIMENTAL OFFICER (MASS SPECTROMETRY)

to assist with the development o analytical methods in biochemica research using a VG 70 - 70F Mas Spectrometer and Finegan Dat System with a Fast-atom bombard ment accessory and other chromatc graphic and analytical equipment Duties involve advising severa research groups in applying thi technique for the characterisatio and assay of biological compound and initiating new analytical pro cedures.

Minimum qualification a firs degree in Chemistry or related topic Candidates who have a higher degre in a relevant area and specific ex perience in mass spectrometry prefer red.

Salary within range £6,310-£8,53 per annum (under review).

Application forms may be obtain ed from the Registrar, The Universit PO Box 147, Liverpool L69 3BX Quote Ref RV/518/N. (2074)A

Please mention nature when replying to these advertisements

UNIVERSITY OF EDINBURGH CENTRE FOR REPRODUCTIVE BIOLOGY

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

LECTURER IN REPRODUCTIVE BIOLOGY

pplications are invited from Scien-sts under 35 years of age for the ove post created under the 'New lood' scheme. Persons already olding a permanent University ap-ointment in the UK are not eligible. he successful applicant will be ex-ected to undertake research related the biology of gametes in col-boration with a team of scientists nd clinicians involved with human vitro fertilization. Teaching sponsibilities include the supervi-on and training of PhD students nd research fellows. There are ex-llent opportunities for col-borative research with other scien-sts in the Centre.

The salary will be at an appropriate oint on the scale £10,710-£14,125 er annum.

Further particulars can be obtain-d from Professor David T Baird, epartment of Obstetrics and ynaecology, University of Edin-urgh, Centre for Reproductive iology, 37 Chalmers Street, Edin-urgh EH3 9EW.

Applications (12 copies) including urriculum vitae and giving the ames and addresses of two referees, hould be sent to the Secretary to the niversity, University of Edinburgh, ld College, South Bridge, Edin-urgh, EH8 9YL, by 30th June, 1984.

Please quote reference NB6(84). (2125)A

UNIVERSITY OF QUEENSLAND Australia

POSTDOCTORAL POSITION — PARASITOLOGY

n immunoparasitology available mmediately to study immunity to astrointestinal nematode parasites n relation to the genetics of the host.

The project is expected to be unded for two years.

Salary per annum: $A24,353. Ref. 7684.

POSTDOCTORAL POSITION AND PROJECT LEADER — PARASITOLOGY (Cattle tick vaccines)

vailable immediately to develop a accine for the control of the cattle ick Boophilus microplus. The roject is funded for two years with n option for extension. Preference o applicants with expertise in mmunology.

Salary per annum $A24,353 with superannuation and reasonable emoval expenses. Ref 18884.

Applications to the Staff Officer, niversity of Queensland, 4067, St ucia, Australia, by June 15, 1984. lease supply names of three referees nd submit a separate application for ach position. (W1009)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London)

ACADEMIC DEPARTMENT OF HAEMATOLOGY

"NEW BLOOD" LECTURESHIP IN MOLECULAR BIOLOGY

Applications are invited for a Molecular Biologist with an interest in the broad field of genetic and acquired haematological disorders. A research group will be formed around a suitable candidate. Some teaching at undergraduate and post-graduate levels will be expected. Major research interests of the Department at present include leukaemia, lymphoma, stem cells, haemophilia and other haemostasis disorders. Post tenable from October 1984.

Salary on scale £7,190-£14,125 (under review) plus £1,186 London Allowance with superannuation benefits. The age of the successful candidate should not normally be over 35 at the date of appointment. Eight copies of curriculum vitae and names of two referees to be sent by 15 June 1984 to the School Office, RFHSM, Rowland Hill Street, London NW3 2PF, (01-794 0500 ext 4262) from which further details may be obtained. (2110)A

UNIVERSITY OF EDINBURGH

POSTDOCTORAL POSITIONS IN MOLECULAR BIOLOGY

Scientists with expertise in immunochemistry/hybridoma technology and gene manipulation are required to join a team engaged in identification and cloning of genes for clinically important antigens of a human malaria parasite.

Send cv and the names of two referees to: Dr J G Scaife, Department of Molecular Biology, Darwin Building, Edinburgh University, King's Buildings, Mayfield Road, Edinburgh EH9 3JR. (1974)A

UNIVERSITY OF ABERDEEN

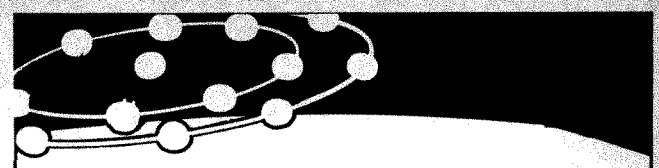
DEPARTMENT OF MICROBIOLOGY

TEMPORARY LECTURESHIP

The Department of Microbiology invites applications for a Temporary Lectureship for one year from October 1984. The post arises from the award of the Nuffield Research Fellowship to Dr I R Booth, and the successful applicant will be expected to assist with teaching in microbial physiology, genetics and molecular biology. There will be the opportunity also to carry through a research programme.

Salary within the range £7,190 to £8,530 per annum, on lecturers salary scale (under review).

Further particulars and application forms from the Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 15 June, 1984. (2139)A



The European Molecular Biology Laboratory, a research institute situated in Heidelberg, West Germany, invites applicants for the following vacancies at the Laboratory's Outstation at the Deutsches Elektronen-synchrotron (DESY) site in Hamburg

APPLICATIONS PROGRAMMER

The successful candidate will help the scientists at the Outstation to design, develop and maintain data acquisition and application programmes for the synchrotron radiation experiments at the Outstation which include protein crystallography, time resolved scattering and X-ray spectroscopy (EXAFS) on biological systems.

He/she will also be expected to take part in the daily operations of the computers at the Outstation: VAX 11/750, PDP 11/45, PDP 11/24. Computer network access is used to maintain collaboration with other groups. Since a large number of projects are carried out by visiting workers, an ability to interact with people is indispensable.

An interest in applying computer technology to scientific problems is indispensable and previous programming experience in a scientific environment will be an asset. The ideal candidate would have at least 3 - 5 years experience using one or more high level languages (FORTRAN, PASCAL). Knowledge of assembler for PDP 11 or VAX is an advantage.

The net salary offered will be around DM 3900, - monthly. Certain allowances are payable in addition, depending on personal circumstances.
An initial contract of 3 years' duration, which can be renewed, will be offered.

Please write briefly for an application form quoting reference N°84/23 to:

EMBL

Personnel Section,
Postfach 10.2209,
D-6900 Heidelberg

(W999)A

POSTDOCTORAL POSITIONS in neurobiology and neuropathology as of 1/7/83. A multidisciplinary-neurobiology group (Drs N K Gonatas, W F Hickey, R Hogue-Angeletti, V M-Y Lee, W W Schlaepfer, J Q Trojanowski) offers positions in a training program to individuals interested in: Endocytosis and Intracellular transport of ligands in vitro and in vivo; Immunological mechanisms mediating EAE; Neurofilament protein structure, Molecular biology, metabolism and expression in vitro and in vivo; molecular mapping of chromaffin granule membrane. Send résumé and names of 3 references to: Dr N K Gonatas, Director, Div of Neuropathology, 447 Johnson Pavilion/G2, University of Pennsylvania School of Medicine, Philadelphia, PA 19104. The University of Pennsylvania is an Equal Opportunity — Affirmative Action Employer. (NW548)A

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Postdoctoral Research Assistantship to work on the isolation and reconstitution of brain D_2 dopamine receptors. The project is funded by MRC and is initially for one year (starting from October 1st, 1984), renewable for a further two years. The salary is on the 1A scale starting at £7,190 per annum.

Applicants should send a curriculum vitae including the names of three referees to Dr P G Strange, Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, as soon as possible. (2133)A

SCIENTIFIC MANAGER

Director Designate **Cheshire**

A small pioneering, yet well established private company, in the field of medical nutrition continues to diversify to satisfy the needs of an expanding market.

You'll have the unique ability to spot the commercial potential in scientific developments and be able to participate as part of the management team in achieving that potential, being totally involved in all aspects of the business.

Aged 35-45, probably a Ph.D, biochemist nutritionist or pharmacist, you'll be thoroughly familiar with lipid biochemistry, clinical trial procedures and have commercial and management experience and capability.

This is a unique opportunity to join a progressive company, offering a very attractive remuneration package at a significant period in its development.

'Phone Dorothy V. Thompson at PER Warrington (0925) 52153.

(2135)A

PER Executive Selection

THE UNIVERSITY OF LANCASTER

Department of Environmental Sciences

The Ionosphere Group of the Environmental Sciences Department has a vacancy for a RESEARCH ASSOCIATE to work on problems of the ionosphere at high latitudes. The project is supported by the Science and Engineering Research Council and the salary will be at an appropriate point on Research Scale IA which commences at £7190 p.a. (currently under review). The appointment is for one year.

Applications are invited from PhD level physicists or geophysicists with interests in radiowave propagation or ionospheric physics.

Further particulars may be obtained (quoting reference L065/ B) from the Establishment Office, University House, Bailrigg, Lancaster, LA1 4YW, where applications (six copies) naming three referees, should be sent NOT LATER THAN 7 JUNE 1984.

(2143)A

Molecular/Cell Biologist

Atomic Energy of Canada Limited invites applications for a staff scientist to join the Radiation Radiology Branch at the Chalk River Nuclear Laboratories. Candidates should possess a Ph.D. degree and preferably have post doctoral experience. This is a continuing position and the successful applicant will be expected to establish an independent research program in an area complementary to the interests of the existing group of ten scientists. The branch research program is internally funded. It focuses on mechanisms of DNA damage and genetic and external factors which influence these processes. Present programs on hyperthermia, mutagenesis, and tumor induction and promotion support these aims. Excellent small animal facilities are available.

Atomic Energy of Canada Limited is a Crown Corporation and first preference will be given to Canadian citizens and landed immigrants of Canada; however, foreign nationals are also invited to apply.

CRNL offers a competitive compensation package and the opportunity to work and live in a pleasant and attractive resort area of the Ottawa Valley, 190 kilometres northwest of Ottawa on the Ottawa River. Good education facilities and year round cultural and recreational facilities are available in the Deep River/Pembroke area.

Qualified women and men should send a complete resumé, including the names of three references and quoting file P-8412 to:

(NW542)A

Employment Supervisor
Atomic Energy of Canada Limited
Chalk River Nuclear Laboratories
CHALK RIVER, Ontario K0J 1J0

Dr. D.K. Myers, Head, Radiation Biology Branch may also be contacted for further information at (613) 584-3311 ext. 2504.

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF ANATOMY AND EMBRYOLOGY

WELLCOME LECTURESHIP

In the Hard Tissue Unit

Tenable for three years in the first instance, which may be renewed for a further two years subject to satisfactory review. The person appointed would be expected to have demonstrated ability for innovative research in the general field of investigation of skeletal & dental tissues and to have had anatomy teaching experience. The candidate shall not be more than 35 years old, or hold a tenured post.

Applications, by June 30th, should include a cv, list of publications, names and addresses of three academic referees and a 500-word summary of the candidate's proposed research.

Salary on Lecturer Scale £7,190-£14,125 (under review) plus £1,186 London Weighting Allowance.

Further information can be obtained by telephone from Professor Alan Boyde or Dr Sheila J Jones. Tel: (01) 387 7050 Ext 635. Applications to be addressed to: Professor A Boyde, Department of Anatomy & Embryology, University College London, Gower Street, London WC1E 6BT. (2129)A

UNIVERSITY OF WALES WELSH NATIONAL SCHOOL OF MEDICINE

TENOVUS INSTITUTE FOR CANCER RESEARCH

RESEARCH TECHNICIAN

required for a period of 3 years (subject to the usual probationary period) to assist in a research programme concerned with hormone binding in prostatic tumours. The work will involve histochemical, biochemical and immunocytochemical techniques for hormone receptor analysis. Applicants should preferably have some experience in biochemistry and/or histology and a degree or equivalent qualification.

Salary on the scale for University Technical Staff Grade 4 (£5,826 — £6,702 per annum) with commencing salary dependent upon qualifications and experience.

Details of the post and application forms available from Dr M E Harper, Tenovus Institute, Heath Park, Cardiff, CF4 4XX. (Tel No (0222) 755944 Ext 2436) to whom applications should be submitted as soon as possible. (2076)A

Please mention

nature

when replying to

these advertisements

UNIVERSITY OF EDINBURGH

FACULTY OF MEDICINE

TEMPORARY LECTURER IN PHARMACOLOGY

Applications are invited for the above post tenable for one year from 1st September, 1984. The appointee will be expected to conduct tutorials, assist in practical classes and lecture to students in the Faculty of Science.

Salary on the scale £7,190-£14,125. USS Superannuation.

Informal enquiries may be made to Professor B L Ginsborg, Department of Pharmacology, 1 George Square, Edinburgh.

Applications (6 copies) including curriculum vitae and the names and addresses of two referees should be sent to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, EH8 9YL, not later than 7th June, 1984.

Please quote reference number 1131. (2142A)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF VETERINARY PHYSIOLOGY

"WELLCOME" LECTURESHIP IN VETERINARY SCIENCE

Applications are invited for a post of Lecturer in the Department of Veterinary Physiology, tenable for a period of three years in the first instance, with possible renewal for a further two years, and available from 1st October 1984. Candidates should be aged 35 or under and should not currently hold a permanent university appointment.

The "Wellcome" lectureships are primarily for research. The appointees will be expected to contribute through fundamental research to understanding of the mechanisms underlying pain and analgesia and to investigate methods for the assessment and alleviation of pain in domestic animals. The successful applicant should bring to this post neurophysiological and neuropharmacological expertise as well as an interest in the welfare of domestic animals.

The initial salary will be at an appropriate point on the lecturer scale £7,190-£12,080 pa.

Applications (3 copies) must include a 500 word summary of the candidate's proposed research, a curriculum vitae with details of research and teaching experience and the names of two referees. Written enquiries should be addressed to Professor A Iggo, Department of Veterinary Physiology, from whom further details may be obtained.

Applications should be submitted to the Secretary of the University, Old College, South Bridge, Edinburgh EH8 9YL before Saturday, 16th June.

Please quote Reference 1126. (2099)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF CLINICAL VETERINARY MEDICINE

Applications are invited for a

LECTURER OR ASSISTANT LECTURER

under the UGC 'New Blood' Scheme. The age limit for 'new blood' appointments is thirty-five, but in exceptional cases candidates just over the age limit may be considered. Preferably applicants should have a veterinary degree, but applications will be considered from applicants with non-veterinary qualifications. The successful candidate will be expected to develop research in respiratory pathophysiology in relation to metabolic consequences of pulmonary damage in farm animals, and to investigate respiratory mechanisms and to correlate structural and functional changes in the lung in relation to the systematic effects of disordered lung function on growth and metabolism. The Department already has ongoing research on the pathogenesis of respiratory disease in calves and the effect of respiratory diseases on pigs.

Tenure: University Lecturer and Assistant Lecturer. Appointment will be for three years with the possibility of reappointment to retiring age in the case of a Lecturer, for an Assistant Lecturer reappointment for two years.

Stipends: University Lecturer: £9,875 rising by eleven annual increments to £15,085: there is no grade of Senior Lecturer.

University Assistant Lecturer: £7,630 rising by four annual increments to £9,425.

Applications should be sent to the Secretary of the Appointments Committee for the Department of Clinical Veterinary Medicine, Madingley Road, Cambridge CB3 0ES, (twelve copies) and should include a curriculum vitae, list of publications, and the names of not more than three referees, to reach him not later than 4 June 1984. (2106)A

UNIVERSITY OF DUNDEE

DEPARTMENT OF PATHOLOGY

POSDOCTORAL RESEARCH ASSISTANT

Applications are invited from cellular immunologists or biologists for appointment for a period of one year in the first instance to work in the Clinical Immunology Laboratory in the above Department on a study of the effect of UVC irradiation of blood on immune competence. Experience in techniques of cellular immunity, tissue culture, flow cytofluorimetry and/or the assay of antibody levels would be useful.

Salary £7190 per annum (under review). Superannuation under Universities Superannuation Scheme.

Further particulars are available from the Personnel Officer, The University, Dundee, DD1 4HN with whom applications containing full career details and the names of 2 referees should be lodged by 14th June 1984. Please quote reference EST/57/84(J). (2116)A

ROYAL MILITARY COLLEGE OF SCIENCE (RMCS) Shrivenham

DEPARTMENT OF MATHEMATICS AND BALLISTICS

RESEARCH SCIENTIST IN COMPUTATIONAL MATHEMATICS

Applications are invited for the post of Research Scientist to work on a project concerned with the computer modelling of fracture problems. The main task of the appointee will be to develop an existing suite of programs into an integrated and flexible software package. The opportunity exists for a suitable candidate to make a significant contribution to research, and it may therefore be possible to register for a higher degree in connection with the work.

The successful candidate is likely to have a good degree with a substantial mathematics and/or computing content. A good knowledge of scientific programming is required and experience in the use of FORTRAN would be an advantage. The person appointed will work in the Department of Mathematics and Ballistics as a member of a team of four within the Computational Mathematics group. Liaison with government research establishments is involved, and opportunities exist for relevant travel, attendance at conferences and publication of results.

The appointment is for a period of one year in the first instance in the grade of Research Scientist (salary scale £5,460 — £8,530 depending on qualifications and experience).

From 1 August 1984, RMCS will become a faculty of the Cranfield Institute of Technology.

Application forms and further information may be obtained from the Civilian Admin Office, Royal Military College of Science, Shrivenham, Swindon, Wilts SN6 8LA. Tel (0793) 782551, ext 421. Please quote reference No HQ 120/1/191.

Closing date for applications 15 June 1984. (2147)A

SOUTHAMPTON AND SOUTH WEST HAMPSHIRE HEALTH AUTHORITY

BASIC GRADE BIOCHEMIST

To work with University Geriatric Medical team based at Southampton General Hospital, linked to Professor Barbara Clayton's department of Chemical Pathology and Human Metabolism. This is a research post, the main interests being bone disease in the elderly and neurochemistry of ageing. Some experience of radioimmunoassay and/or receptor-binding techniques would be an advantage.

Salary Scale (post probationer £6,520 — £8,349). Application form and job description from District Personnel Department, Tremona Court, 30/32 Tremona Road, Southampton. Telephone Southampton 777222 ext 3018/3556. Closing date 8 June 1984. (2149)A

Nestlé

Our Central Research Department in Vevey/Switzerland is seeking a

chemist or biochemist

to work in the protein field.

Activity

- Analysis of food proteins by electrophoretic and chromotographic techniques.
- Extraction, fractionation and purification of proteins from various raw materials on laboratory and pilot plant scale.
- Study of physicochemical and functional properties of food proteins.

Qualifications required

- University degree with Ph.D.
- Experience in protein purification and characterization.

Please send your application for employment with curriculum vitae and photograph to:

Miss Myriam Germanier
Personnel Services,
Nestlé Products Technical Assistance Co. Ltd.,
1800 Vevey / Switzerland. (W997)A

Nestlé

UNIVERSITY OF CAPE TOWN

Senior Lecturer in Physical Oceanography

Applications are invited for the above post, vacant from 1 July 1984.

Candidates should have interests in the broad field of dynamical oceanography, those with interests in air-sea interaction and/or marine climatology being particularly welcome. The successful applicant will be required to take part in undergraduate and postgraduate teaching and to undertake research.

Appointment, according to qualifications and experience will be made on the salary scale R16 557 x 936–24 045 plus a pensionable allowance of 12%, with an annual bonus of nearly one month's salary and attractive staff benefits.

Applicants should submit a full curriculum vitae and the names and addresses of three referees not later than 15 June 1984 to the Registrar, (Attention: Appointments Office) Department AC/452, University of Cape Town, Private Bag, Rondebosch, 7700, South Africa. Further information may be obtained from the Registrar or The Secretary, SA Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE.

The University's policy is not to discriminate on the grounds of sex, race or religion. Further information on the implementation of this policy is obtainable on request.

FSR 0641 (W1001)A

Protein Chemist

G.D. Searle is a major international pharmaceutical company with a strong commitment to Research & Development in the UK. The Company is actively involved in the fields of chemically and biologically derived medical products.

A vacancy has arisen for an experienced Protein Chemist to join a group working on the purification and characterisation of genetically engineered proteins. Applications are invited from post graduates with a minimum of 3 years relevant experience which ideally would include specialised experience of protein structure and folding.

The Company offers:—

* 37½ hour working week
* 23 days basic holiday entitlement
* subsidised restaurant
* generous health insurance
* contributory pension scheme
* active recreation club

Please apply to Miss D. Wardman, Assistant Personnel Officer, Searle Research & Development, Lane End Road, High Wycombe, Bucks (Tel: High Wycombe 21124, ext 3254). Please quote reference PR21. (2103)A

SEARLE

KING'S COLLEGE SCHOOL OF MEDICINE & DENTISTRY
King's College London
Denmark Hill
London SE5 8RX

DEPARTMENT OF OBSTETRICS & GYNAECOLOGY

"NEW BLOOD" LECTURESHIP NON-CLINICAL MILECULAR GENETICS IN HUMAN REPRODUCTION

Applications are invited for the above post which will be tenable in the Harris Birthright Research Centre for Fetal Medicine in the Department of Obstetrics and Gynaecology. Candidates (normally under 35 years of age) should have a higher degree and experience in recombinant DNA technology. The successful applicant will be expected to apply these methods to the department's current research on pre-natal diagnosis and in vitro fertilisation and also contribute to teaching.

Salary on the scale for non-clinical lecturers £7190-£14,125 plus £1186 London Weighting Allowance per annum.

Further details may be obtained from the Secretary, King's College School of Medicine & Dentistry, Denmark Hill, London SE5 8RX, to whom applications, giving the names and addresses of three referees, should be sent no later than Thursday 14 June 1984. (2089)A

SCOTTISH UNIVERSITIES RESEARCH AND REACTOR CENTRE

POST OF DIRECTOR

The Centre was opened in 1963 to provide research and teaching facilities in the reactor field for the Scottish Universities. Since then the range of its activities has widened to include isotope geology and nuclear medicine.

The Directorship of the Centre will become vacant on the retiral of Professor H W Wilson on 30th September, 1985. Applications are invited from suitably qualified persons for this post which is on the professorial salary scale. The successful candidate will probably be a physicist, chemist, isotope geologist or engineer with experience in the broad areas of reactor science and engineering, isotope geology or nuclear medicine, in all of which fields the Centre has an established reputation. The Director will be expected to be the scientific leader of the Centre.

Further particulars may be obtained from the Academic Personnel Office, University of Glasgow, Glasgow G12 8QQ, where applications (3 copies; 1 copy in the case of overseas applicants), giving the names and addresses of not more than three referees, should be lodged on or before 30th June, 1984.

In reply please quote Ref No 5256M. (2124)A

SOUTHAMPTON AND SOUTH WEST HEALTH AUTHORITY

SENIOR MEDICAL PHYSICS TECHNICIAN
in the
DEPARTMENT OF REHABILITATION

SENIOR MEDICAL PHYSICS TECHNICIAN
(Whitley Council Grade 2)

required to work in University Clinical Rehabilitation laboratories. The research work will involve the recording and processing of biological potentials from patients with neurological disease. The post includes provision of technical assistance to research students, the design and construction of electronic processing equipment, the supervision of other research staff and responsibility for safety and quality assurance in the laboratory. Previous experience in laboratory supervision and clinical projects will be an advantage.

Application form and job description from District Personnel Department, Tremona Court, 30/32 Tremona Road, Southampton. Telephone Southampton 777222 ext 3018/3556. Closing date 8 June 1984. (2150)A

KING'S COLLEGE LONDON
DEPARTMENT OF BIOPHYSICS

GRADE 3 TECHNICIAN

for a research group concerned with molecular mechanisms of cell motility and growth related to the cytoskeletal system of cultured cells.

This appointment, funded by the Medical Research Council, is for three years from October 1984. Salary £6,401-£6,689 inclusive.

Please apply in writing with the names and addresses of two referees by 15 June 1984 to Dr A Koffer, King's College London, 26-29 Drury Lane, London WC2B 5RL. (2126)A

IMPERIAL COLLEGE
DEPARTMENT OF CHEMISTRY AND CENTRE FOR BIOTECHNOLOGY

Applications are invited for

2 POST-DOCTORAL RESEARCH ASSISTANTS
and for
2 SERC STUDENTSHIPS

starting from 1 October 1984 to work with Professor W J Albery, Dr J A Barrie, Dr A E G Cass and Dr M H George on the development of enzyme electrodes and their associated membranes.

Applications including a curriculum vitae and the names of two referees should be sent as soon as possible to Professor W J Albery, Department of Chemistry, Imperial College, London SW7 2AY. (2122)A

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

Applications are invited for a thr year appointment as

POST-DOCTORAL ASSISTANT

to carry out a fundamental study the role of agitation in suspensio polymerisation reactors. The wor will be under the direction of Dr G Maitland and Dr S M Richardson, i collaboration with ICI Petro chemicals and Plastics Division. Th appointment will be from Autum 1984 or earlier by mutual agreemen Applicants should have experience experimental research in engineerin or the physical sciences; a bac ground in fluid mechanics, rheolog or polymerisation work would be particular advantage. Starting sala in range £7,190-£8,530 pa plus £1,18 London Allowance, according to ag and experience.

Applicants should send CV an names of two referees to Dr G Maitland, Department of Chemica Engineering and Chemical Tech nology, Imperial College, Londo SW7 2BY, from whom further info mation may also be obtained. (2102)A

THE UNIVERSITY OF BIRMINGHAM

FACULTY OF MEDICINE AND DENTISTRY

CLINICAL GENETICS

A vacancy has arisen in the abo department for a Research Associa to work on cytogenetic aspects mental retardation.

Salary — Research Associate £6310 — £8530 (plus superannu tion) for two years.

Further information from tant Registrar, The Medical Sch Birmingham B15 2TJ, to whom plications (three copies) must be s by 15th June 1984. Quote R RA/CG/TW. (2096)A

UNIVERSITÄT INNSBRUC

BIOCHEMISCHE PHARMAKOLOGIE

Am Lehrstuhl für Biochemisc Pharmakologie der Medizinisch Fakultät sind ab sofo Universitätsassistentenstellen besetzen.

Arbeitsgebiet: Biochemisc Charakterisierung des Calciu kanals.

Mediziner, Pharmazeute od Biochemiker mit entspre Ausbildung und Motivation werd um Kurzbewerbung (Publikation verzeichnis, Referenzen) gebeten.

Prof Dr H Glossmann, Ordinari für Biochemische Pharmakologi Fritz-Preglstr 3, A-6020 Innsbruc Austria. (W1014)A

UNIVERSITY OF LONDON
Royal Postgratduate Medical School

WELLCOME LECTURESHIP IN VIROLOGY

Applicatons are invited from candidates trained in the techniques of modern molecular biology, such as the M13 (Sanger) cloning sequencing system, site-specific mutagenesis, and molecular cloning, for a new post of Wellcome Lecturer in the Department of Virology. The successful candidate will be required to develop the application of molecular biological techniques in a clinical environment and to contribute to the teaching activities of the Department.

Candidates should be not more than 35 years of age and must not hold a permanent university appointment in the United Kingdom. Appointment will be for three years in the first instance with a possible extension for a further two years. Salary on the non-clinical lecturer scale, currently £7,190 — £14,125 plus £1,186 London Allowance.

Applicants should apply by letter, enclosing 6 copies of their curriculum vitae, a statement of not more than 500 words on the research they would propose to undertake if appointed, and naming three referees to: The Deputy Secretary, Royal Postgraduate Medical School, Hammersmith Hospital, DuCane Road, London W12 0HS, from whom further particulars can be obtained (telephone: 01-743 2030, ext. 263).

Closing date for applications: 15 June 1984. (2085)A

THE UNIVERSITY OF SYDNEY
DEPARTMENT OF BIOCHEMISTRY

LECTURESHIP

Duties will consist of teaching and research. The Department teaches Biochemistry to students in the following courses: Dentistry, Medicine, Science, Pharmacy and Veterinary Science. Applications are invited from persons suitably qualified in Biochemistry: preference may be given to those with special interest in the area of th eukaryotic gene structure and expression.

Salary range: $A23,394 - $A30,734 pa (under review).

Appointments to Lectureships are usually probationary for three years and the University reserves the right not to proceed with any appointment for financial or other reasons.

Applications, quoting reference no 18/07 and including curriculum vitae, list of publications and names of three referees to the Registrar, University of Sydney, NSW 2006 Australia by 15 June 1984, from whom further information available. Further general information also available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (W1004)A

UNIVERSITY OF CAMBRIDGE

LECTURER IN THE DEPARTMENT OF METALLURGY AND MATERIALS SCIENCE

To take up appointment of 1 October 1984 or as soon as possible thereafter. Preference will be given to candidates with research experience in the science and engineering aspects of composite materials. The lecturer will be expected to teach in the broad field of non-metallic materials.

The appointment will be for three years with the possibility of reappointment to the retiring age. The pensionable scale of stipends for a University lecturer, not ordinarily resident in college, is £9875 rising by eleven increments to £15085.

Further information about the duties and conditions of appointment may be obtained from the Secretary of the Department of Metallurgy and Materials Science, Pembroke Street, Cambridge CB2 3QZ. Applications, including the names of three referees, should be sent to Secretary of the Faculty of Physics and Chemistry, Institute of Astronomy, Madingley Road, Cambridge, so as to reach him not later than 27 June 1984.

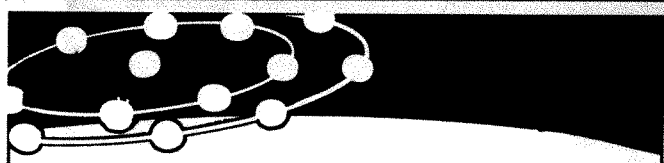
(2145)A

UNIVERSITY OF DUNDEE
WELLCOME LECTURESHIP IN PHYSIOLOGY

PHYSICAL CHEMIST/ BIOCHEMIST/ PHYSIOLOGIST

Applications are invited for the above full-time post tenable in the Department of Physiology, University of Dundee, for up to five years from September 1984. Candidates must be under 35 years of age and not presently hold a permanent position in a University or research establishment within the United Kingdom. A condition of appointment is that, besides carrying out teaching to Medical and Science students, the successful candidate will be expected to work with Professor M J Rennie in the general area of physiological metabolism on the development and application of stable-isotope-labelling techniques suitable for use in studies of human physiology and medicine. Preference will be given to candidates with previous experience of stable-isotope methodology who can demonstrate an ability to apply this expertise to physiological problems or to physiologists who are willing to make themselves expert in methodologies involved.

Further particulars are available from the Personnel Officer, the University, Dundee, DD1 4HN (telephone 0382 23181 extension 383) and prospective applicants are requested to obtain these before submitting formal applications and outlines of the research projects they have in mind to undertake with Professor Rennie. Please quote reference EST/17/84(J). (2115)A



The European Molecular Biology Laboratory, a research institute situated in Heidelberg, West Germany, invites applicants for the following vacancies at the Laboratory's Outstation at the Deutsches Elektronen-synchrotron (DESY) site in Hamburg.

PROTEIN CRYSTALLOGRAPHER

The Outstation has developed advanced instrumentation for the use of synchrotron X-ray radiation in structural biology Protein Crystallography, fibre and solution diffraction, X-ray spectroscopy (EXAFS). The facilities are used by many groups mainly from the Member States. The successful candidate will be expected to engage in research projetcs, making use of the outstanding properties of synchrotron radiation (high intensity, wavelength tunality, polarization and time structure) for protein crystallography. Besides these activities, she/he will also assist outside groups with protein crystallographic data collection.

The ideal candidate would be a protein crystallographer with 3-5 years of experience, capable of carrying out the various aspects of a crystallographic project. Fluent knowledge of English is a prerequisite.

The net salary offered will be between DM 3900, - and DM 4700, - monthly. Certain allowances are payable in addition, depending on personal circumstances. An initial contract of 3 years' duration, which can be renewed, will be offered.

Please write briefly for an application form quoting reference N°84/24 to :

EMBL

Personnel Section,
Postfach 10.2209,
D-6900 Heidelberg

(W1000)A

UNIVERSITY COLLEGE OF LONDON
DEPARTMENT OF CHEMICAL AND BIOCHEMICAL ENGINEERING

A POST-DOCTORAL MEMBRANE BIOCHEMIST/ MICROBIOLOGIST

is sought to join a team supported by a Special Rolling Grant from the Science and Engineering Research Council. The programme is concerned with biochemical engineering studies to improve the processing of proteins and enzymes, and one of its objectives is to facilitate the large-scale isolation of membrane-associated enzymes. This project has been under way for five years and is presenting the biochemical engineers and bioscientists involved with new and exciting challenges. The present phase of work is funded until 1987. The team is part of a larger, internationally respected group of UCL engaged in enzyme, fermentation and protein technology studies and which has strong external links to relevant biochemistry, microbiology and genetics research teams.

Salary in range £7190 — £9425 per annum plus £1186 per annum London Allowance.

Applications should be addressed to Dr Peter Dunnill, Department of Chemical and Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, England. (2084)A

UNIVERSITY OF PENNSYLVANIA
School of Medicine
DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE

SENIOR CELLULAR AND/OR MOLECULAR IMMUNOLOGIST

Applications are invited for a senior, tenured faculty position to head the Division of Research Immunology in the Department of Pathology and Laboratory Medicine.

The individual sought should possess a record of research achievement in cellular and/or molecular immunology and have demonstrated capability in research training. This person will be expected to continue the development of this established, nationally recognized research and training program, including recruitment of new members. No clinical service responsibilities are required of this position.

Interested candidates should submit résumés and names of three references prior to July 15, 1984 to Drs Leonard Jarett and Peter C Nowell, Co-Chairmen, Immunology Search Committee, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104.

The University is an Affirmative Action/Equal Opportunity employer. (NW549)A

ST. GEORGE'S UNIVERSITY SCHOOL OF MEDICINE

GRENADA ST.VINCENT BARBADOS

St. George's University School of Medicine, located in the West Indies, is expanding its present faculty. Applications are invited from PhDs or MDs.

Teaching positions are available at the level of instructor, assistant Professor or Associate Professor, in the following subjects: Biochemistry, Microbiology. Lecturers and laboratory demonstrators in Pathology, Pharmacology, Physiology, Histology.

Recent retirees in good health, and experienced lecturers available during a sabbatical leave of at least one year will also be considered. Priority will be given to applicants with prior experience in lecturing in Medical Schools. These faculty appointments will begin July or August 1st 1984.

Submit applications including current curriculum vitae and recent references to the Vice Chancellor, St. George's University School of Medicine, c/o 849 Lullwater Parkway, Atlanta GA. 30307, U.S.A. (W1002)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)

POSTDOCTORAL PHYSIOLOGIST

A three year MRC funded post for a postdoctoral candidate with a background in mammalian physiology will become available from October 1 1984, to work on circulatory reflexes with Professors M de B Daly and K M Spyer.

Salary on Scale Research 1A.

Further details are available from the School Office, Room G650, RFHSM, Rowland Hill Street, London NW3 2PF or telephone 01-794 0500 Extn 4262, to which application by full curriculum vitae, including the names and addresses of two referees, should be submitted by 8 June 1984. Please quote reference: S/RA. (2131)A

RESEARCH ASSOCIATE
Post Doctoral

Cell biology or biochemistry. Basic studies on intracellular peptide processing, glycosylation, transport and secretion. 2-3 year appointments. Salary $20,000.

David V. Cohn, PhD Departments of Oral Biology and Biochemistry, Health Sciences Center, University of Louisville, Louisville, KY 40292. USA (502) 588-7507. (NW543)A

UNIVERSITY COLLEGE DUBLIN

Applications are invited by the Governing Body of the College for the following full-time statutory appointment:

PROFESSORSHIP OF PHYSIOLOGY AND HISTOLOGY

Prior to application, further information (including application procedure) may be obtained from the Secretary and Bursar, University College, Belfield, Dublin 4. Telephone enquiries: 693244 ext. 431.

The closing date for receipt of completed applications is Thursday, 21st June 1984. (2088)A

HOWARD HUGHES MEDICAL INSTITUTE
DEPARTMENT OF PHARMACOLOGY

RESEARCH ASSOCIATE POSITION AVAILABLE

Research program is in the area of molecular basis of calcium action, especially the mechanism of action of the calmodulin family of proteins. Background in Molecular Biology or Biochemistry preferred, but motivation and ability to interact with a multidisciplinary group more important.

Send curriculum vitae and names of two references to D Martin Watterson, Department of Pharmacology and Howard Hughes Medical Institute, 723 Light Hall, Vanderbilt University, Nashville TN 37232. (NW547)A

LUDWIG INSTITUTE FOR CANCER RESEARCH
LONDON BRANCH
Haddow Laboratories,
Clifton Avenue
Sutton, Surrey SM2 5PX

POSTDOCTORAL CHEMIST

Applictions are invited from a Postdoctoral Biochemist for a research post in a group studying the biology of the human breast. The post will involve predominantly the characterisation of intermediate filaments in a human breast epithelial cells and the successful applicant will be a member of an integrated team of biologists, membrane biochemists and pathologists investigating differentiation in this system. Practical experience of protein separation techniques including two dimensional gel electrophoresis is essential. Tissue culture experience would be an advantage but not essential as the opportunity to learn this and monoclonal techniques would be provided in the course of the project.

The post will be classified as Non-Clinical Scientist Grade II (MRC Scale) and salary range within £7,190-10,710 pa plus £1,1865 pa London Weighting.

Applications should be sent in writing, enclosing curriculum vitae and the names and addresses of two referees to the Administrator at the above address before 8th June 1984. (2083)A

THE PAPUA NEW GUINEA UNIVERSITY OF TECHNOLOGY

LECTURER IN DEPARTMENT OF APPLIED PHYSICS

Applications are invited for the post of Lecturer in the Department of Applied Physics.

The appointment is of 3 years duration during the absence of a national staff member on study leave.

The appointee will be expected to teach general physics in service courses in addition to advanced teaching and research. A qualification in geophysics is preferred.

Salary: Lecturer I K16,820 per annum; Lecturer II K18,670 per annum (K1 = Stg 0.8209) (Level of appointment will depend upon qualifications and experience).

The initial contract period is for approximately three years. Other benefits include a gratuity of 24% taxed at 2%, appointment and repatriation fares, leave fares for the staff member and family after 18 months of service, settling-in and settling-out allowances, six weeks paid leave per year, education fares and assistance towards school fees, free housing, salary protection plan and medical benefit schemes are available.

Detailed applications (two copies) with curriculum vitae, together with the names and addresses of three referees and indicating earliest availability to take up appointment should be received by: The Registrar, Papua New Guinea University of Technology, Private Mail Bag, Lae, Papua New Guinea, by 15 June 1984.

Applicants resident in the United Kingdom should also send one copy to Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF, from whom further general information may be obtained. (W1010)A

CHARING CROSS HOSPITAL MEDICAL SCHOOL
(University of London)

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for this post in the Department of Biochemistry to work on an MRC-funded project on the effect of oestrogens on proteoglycan synthesis in connective tissue cells. Applicants should have obtained a PhD degree and preferably have some experience in cell culture techniques.

Starting salary £7,190-£7,630 pa plus £1,186 pa London Allowance.

Informal enquiries to Dr R Mason, 01-748 2040 ext 2604/2841.

Applications should include a full curriculum vitae and the names and addresses of two referees, to be sent to The Secretary, Charing Cross Hospital Medical School, The Reynolds Building, St Dunstan's Road, London W6 8RP, by 20th June. (2132)A

THE UNIVERSITY OF BIRMINGHAM
FACULTY OF MEDICINE AND DENTISTRY
DEPARTMENT OF CANCER STUDIES

POST-DOCTORAL CELL BIOLOGIST/ IMMUNOLOGIST

A vacancy exists for a post-doctoral scientist with experience in cell biology/immunology to join a research group studying cellular transformation by the Epstein-Barr virus. The project, which will be carried out in the Department's new laboratory, is funded for up to three years by the Cancer Research Campaign. The position is available immediately, although consideration may be given to applicants who are completing postgraduate work later this year. Further details are available from Professor A B Rickinson of the above Department (021) 472 7607.

Salary on scale Research Fellow 1A £7,190-£11,615 with superannuation.

Applications (three copies) with names of two referees to Assistant Registrar, Medical School, Birmingham, B15 2TJ by 14th June 1984. Quote ref: RF/CS/Imm. (2095)A

UNIVERSITY OF LONDON
CHAIR OF PHARMACOLOGY AT THE UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST THOMAS'S HOSPITALS

The Senate invite applications for the above post. Applications (11 copies) should be submitted to the Academic Registrar (N), University of London, Malet Street, London WC1E 7HU, from whom further details should first be obtained. Closing date for receipt of applications is 8 June 1984. (2101)A

UNIVERSITY OF NOTTINGHAM
SCHOOL OF AGRICULTURE
Sutton Bonnington,
Nr Loughborough

TECHNICIAN GRADE 3

Applications are invited for the above post within the Department of Physiology and Environmental Science.

The work mainly involves the assessment of hormone-producing capacity of ovarian tissue under various physiological conditions using radioimmunoassay techniques, preparation of ovarian tissue for histology and assistance with in vitro tissue culture. Maintenance of a small suite of laboratories serving a Research Group is part of the routine duties.

This research funded post for one year in the first instance, and possibly renewable, has a current salary scale £5,151 to £6,035 per annum for a 5-day, 37-hour working week.

Write or telephone the Assistant Registrar at the School (Kegworth 2386) for an application form quoting Post Ref. 84/7. (2112)A

THE UNIVERSITY OF BIRMINGHAM
FACULTY OF MEDICINE AND DENTISTRY
DEPARTMENT OF CANCER STUDIES
POST-DOCTORAL MOLECULAR BIOLOGIST/ VIROLOGIST

A vacancy exists for a post-doctoral scientist with experience in molecular biology/virology to join a research group studying cellular transformation by the Epstein-Barr virus. The project, which will be carried out in the Department's new laboratory, is funded for up to three years by the Cancer Research Campaign. The position is available immediately, although consideration may be given to applicants who are completing postgraduate work later this year.

Salary on scale Research Fellow 1A £7,190-£11,615 with superannuation.

Further details are available from Professor A B Rickinson of the above Department (021) 472 7607.

Applications (three copies) with names of two referees to Assistant Registrar, Medical School, Birmingham, B15 2TJ by 14th June 1984. Quote ref: RF/CS/Vir. (2094)A

POSTDOCTORAL RESEARCH POSITION: Available immediately for recent PhD or MD with a strong background in molecular biology/genetics of animal viruses. Candidates with excellent academic records, some experience in DNA technologies including cloning and a strong interest in tumor virology will be given preference. Please send résumé, abstract of the thesis along with other evidence of scholarly productivity, and the name and telephone numbers of three referees to: Nurul H Sarkar, PhD, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, NY 10021, USA. (NW541)A

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY
BIOPHYSICS SECTION
RESEARCH OFFICER

for protein crystallization studies. Our work on the crystal structure of enzymes, especially of modified enzymes created by protein engineering, demands greater effort in protein crystallization. We seek an applicant with an understanding of the properties of protein molecules in solution, and with wide practical experience in protein chemistry. A good honours degree in Biochemistry, or the equivalent, and some years postgraduate experience in relevant work are expected. An applicant for part-time work would be considered.

The appointment will be in the ORS IB range £6310-£9875 + £1186 London Allowance, and will be funded by MRC to August 1987 in the first instance.

Applications, including a CV and the names of two referees, should be addressed to Professor D M Blow, FRS, Blackett Laboratory, Imperial College, London SW7 2BZ. (2120)A

MONASH UNIVERSITY
Melbourne, Australia
DEPARTMENT OF BIOCHEMISTRY
FIXED-TERM LECTURER (3-5 YEARS)

Postdoctoral graduate to teach science or medical students and participate in ongoing research programme into physical biochemistry of connective tissues. Candidates should have a general background in biochemistry/chemistry with research experience in techniques of physical biochemistry. Applicants with experience in polymer research preferred, but this is not essential.

Enquiries to Professor A W Linnane.

Salary: $A24,353—$A31,994 pa.

Applications including Ref no 30212, curriculum vitae and 3 referees to the Registrar, Monash University, Clayton, Vic 3168, Australia, with a copy to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, by 1/8/84, from whom Conditions of Appointment available.

An equal opportunity employer. (W1003)A

THE UNIVERSITY OF NORTH CAROLINA
DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
RESEARCH ASSOCIATE

Available immediately for postdoctoral scientist to participate in studies of pathogenic RNA virus gene expression and function using recombinant DNA technology.

Send curriculum vitae and 3 letters of reference to Dr G W Wertz, Department of Microbiology & Immunology, University of NC, Chapel Hill, NC 27514.

The University of NC is an Equal Opportunity/Affirmative Action Employer. (NW546)A

UNIVERSITY OF WARWICK
LECTURESHIP IN BIOTECHNOLOGY (PLANT BIOCHEMISTRY)

Applications are invited for a lectureship in the Department of Biological Sciences from persons with a PhD in biochemistry, genetics, or related subjects interested in applying modern biochemical techniques, especially recombinant DNA technology, to study the expression of genes for plant proteins especially with the aim of producing mutant forms of toxic and allergenic proteins. The appointee will be expected to develop his or her own research programmes and to teach in undergraduate courses. Initial salary will normally be within the first six points of the Lectureship scale: £7,190 — (£9,425) — £14,125 pa. Further particulars and application forms from the Registrar, University of Warwick, Coventry CV4 7AL quoting Ref. No: 42/A/84/O. Closing date for receipt of applications 15th June, 1984. (2148)A

ASSISTANTSHIPS

UNIVERSITY OF GLASGOW
Department of
Electronics and Electrical Engineering

Research Assistantships (JOERS)

Applications are invited for three Research Assistantships supported by S.E.R.C. on research grants awarded under the Joint Optoelectronics Research Scheme.

1. **$LiNbO_3$ Integrated Optical Devices:** to work on novel optoelectronic switching and modulation devices for operation at microwave and millimetre wave frequencies, with applications in high-capacity optical fibre telecommunications and microwave radar systems.
2. **Modular Beam Epitaxy of III-V Semiconductors:** for applications in advanced optoelectronic device structures.
3. **Coherent Optical Signal Processing:** applied to the homodyne receiver. Modelling, theoretical analysis and experimental assessment of various types of optical homodyne phase-locked loops using frequency, phase and amplitude modulated signals.

Those appointed will join an established research group with excellent support facilities, and will work in close co-operation with the academic and industrial partners in each JOERS project. Applicants should possess a Ph.D. or have equivalent research experience in electronic engineering, physics or materials science. Starting salary up to £9,875 (under review) on the 1A scale for research staff. The posts are tenable for two or three years in the first instance, subject to satisfactory progress. Applications (3 copies) stating qualifications and experience and naming two referees, to: Dr. P.J.R. Laybourn, Department of Electronics and Electrical Engineering, University of Glasgow, G12 8QQ from whom further particulars of the posts are available. (2134)P

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

Applications are invited for a
POST-DOCTORAL RESEARCH ASSISTANTSHIP

in molecular biology for work on the development of plant gene vectors from virus replicons, involving DNA cloning and sequencing, transcriptional involving DNA cloning and sequencing, transcriptional mapping, in vitro translation and plant cell transformation. The appointment will be for 2 years from 1 October 1984 with the possibility of renewal for a further period. Salary in the range £8376-£9716 including London Allowance.

Applications, with names of two referees, to Dr K W Buck and Dr R H A Coutts, Department of Pure and Applied Biology, Imperial College, London SW7 2BB. (2121)P

FELLOWSHIPS

UNIVERSITY OF FREIBURG

Applications are invited for a
POSTDOCTORAL FELLOWSHIP IN NEUROCHEMISTRY

to study metabolism of neuropeptides in CNS and CSF.

Curriculum vitae and references should be sent to: Dr H Cramer, Professor, Section on Neurochemistry, Department of Neurology, Hansastr 9, D-7800 Freiburg, West Germany. (W1007)E

FRIEDRICH MIESCHER-INSTITUT
Basel, Switzerland

POSTDOCTORAL FELLOWSHIP IN PROTEIN BIOCHEMISTRY

Applications are invited for a postdoctoral position to study protein interactions involved in blood coagulation. It is envisaged that a wide range of techniques will be used, e.g. protein modification, binding and kinetic studies. Experience in protein purification together with protein chemistry of enzymology is desirable.

The position is available immediately for a period of one year with the possibility of renewal for a further two years.

Applications, including curriculum vitae and the names of two referees should be sent as soon as possible to Dr. S.R. Stone, Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland. (W1008)E

Continued on page 14

FELLOWSHIPS continued

UNIVERSITY OF GUELPH

UNIVERSITY FELLOWSHIPS

The College of Biological Science is enlarging its Research and Graduate Programs and invites applications from individuals interested in competing for NSERC University Fellowships. Candidates are reminded that they would be eligible for consideration for any tenure track position available following their appointment.

BOTANY
Growth and development of plants

MICROBIOLOGY
Bacterial cell envelope structure and function
Physiology of fish pathogens
Fermentation technology
Molecular biology of virus reproduction

MOLECULAR BIOLOGY & GENETICS
Cell and molecular biology of gene expression
Developmental biology
Genetic recombination

NUTRITION
Nutritional immunology
Nutritional toxicology
Food toxicology

ZOOLOGY
Comparative physiology particularly in marine systems
Fisheries ecology

SCHOOL OF HUMAN BIOLOGY
Human physiology
Physical anthropology
Ergonomics
Human biomechanics/bioengineering

Successful candidates would have the opportunity of participating in both the undergraduate and graduate teaching programs.

Applications, including a curriculum vitae, and the names of three referees should be forwarded, by August 1st, to:

Bruce H. Sells, PhD
Professor and Dean
College of Biological Science
University of Guelph
Guelph, Ontario
Canada N1G 2W1

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.

(NW545)E

UNIVERSITY OF EAST ANGLIA
Norwich

RESEARCH FELLOWSHIP IN CHEMISTRY (NUCLEAR MAGNETIC RESONANCE)

Applications are invited for a two-year Research Fellowship in nuclear magnetic resonance, to be held in the School of Chemical Sciences. Applicants should have experience of modern NMR methods of post-doctoral level. The emphasis of that experience should be in physical chemistry. The post will also involve some lecturing duties. The post is on the Research and Analogous 1A scale, at or below the fourth point (currently £8530 per annum).

Applications should be made to Professor R K Harris, School of Chemical Sciences, University of East Anglia, Norwich NR4 7TJ, and should include a curriculum vitae, list of publications, and the names and addresses of two academic referees. The closing date for applications is 30 June 1984. The appointment may commence on 1 October 1984.

(2141)E

ESF PROGRAMME OF RESEARCH FELLOWSHIPS IN TOXICOLOGY (PGT)

Short-term (up to 6 months) and Long-term (6 to 12 months) Research Fellowships (deadline: 30 June 1984) and Short-term (up to 1 month) Visiting Fellowships (no deadline) are offered to scientists working in European laboratories to go to laboratories in other European countries. No condition of age, level or field of training, provided the project contributes to furthering research in TOXICOLOGY in Europe.
Information and application forms can be obtained from: Dr. Bernard Munsch, European Science Foundation, 1, quai Lezay-Marnésia, F-67000 STRASBOURG, France; tel. (88) 35.30.63; telex 890440.

(W1005)E

Please mention
nature
when replying to
these advertisements

STUDENTSHIPS

UNIVERSITY OF NOTTINGHAM

SERC RESEARCH STUDENTSHIP IN PLANT ULTRASTRUCTURE

Applications are invited for a SERC Quota Research Studentship, to work on the ultrastructure of embryogenesis and seed formation in Arabidopsis, in the Department of Botany under the supervision of Dr L G Briarty, starting on October 1, 1984. The work forms part of a project being carried out with the European Space Agency on the effects of microgravity on cell ultrastructure and development.

Applicants should have, or expect to obtain, a first or upper second class honours degree in Botany, Biology or a related field, and some experience of electron microscopy. Requests for further information, and applications with the names of two academic referees, should be sent as soon as possible to Dr L G Briarty, Department of Botany, University of Nottingham, University Park, Nottingham NG7 2RD. (2100)F

UNIVERSITY OF STIRLING
DEPARTMENT OF BIOLOGICAL SCIENCE

SERC CASE STUDENTSHIP

Applications are invited for a three year PhD studentship to work on the changes of stomatal behaviour during the course of a growing season. The study will involve some field work and also physiological and biochemical investigations. The award is held co-operatively with Dr H G Jones of East Malling Research Station and will commence in October 1984.

Applications, enclosing a curriculum vitae and the names of two referees should be sent to Dr C M Willmer, University of Stirling, Department of Biological Science, Stirling FK9 4LA. (2137)F

UNIVERSITY OF OXFORD
DEPARTMENT OF HUMAN ANATOMY

MRC RESEARCH STUDENTSHIPS

Applications are invited from students possessing, or expecting to possess, a good Honours (First or Upper Second) degree for studentships in the following areas:

1) Intracellular calcium and the regulation of solute transport by small intestine (Dr C A R Boyd)

2) An analysis of gonadotrophic hormone gene expression in mutant mice using recombinant DNA technology (Dr H M Charlton)

3) Pattern formation in the developing nervous system (Dr T J Horder)

Applicants should forward a letter of application with a full curriculum vitae and the names of two referees to Dr S Bradbury, Acting Head of Department, Department of Human Anatomy, South Parks Road, Oxford OX1 3QX by Monday, 4 June. Interviews of selected candidates will be held on Wednesday, 13 June. (2114)F

UNIVERSITY OF NOTTINGHAM
SCHOOL OF AGRICULTURE
Food Research

Applications are invited for

TWO RESEARCH STUDENTSHIPS

(1) A CASE studentship to work on protein/polysaccharide interactions in extruded food products.

(2) A MAFF sponsored studentship concerned with the relationship between the viscoelastic behaviour and mouthfeel of modified starch solutions.

The people appointed will form part of a multidisciplinary team working on various aspects of food biopolymers.

Applicants should have or expect to obtain a first or upper second class honours degree in chemistry, food science, physics or biochemistry.

If you are interested, please write to: Dr J R Mitchell, Department of Applied Biochemistry & Food Science, School of Agriculture, University of Nottingham, Sutton Bonington, Loughborough LE12 5RD giving details of academic record and the names of two referees. It would be helpful if you state your preferred research topic. (2111)F

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOCHEMISTRY

MRC/SERC POSTGRADUATE RESEARCH STUDENTSHIPS FOR OCTOBER 1984

Following an increased number of awards from both the MRC and SERC, the Department invites further applications in one of the following areas from persons who expect to graduate with at least an upper Second Class Honours Degree this Summer.

1. Role of protein phosphorylation in Insulin and Growth factor action; Cloning of protein kinases and protein phosphatases (Dr D G Hardie, Dr P T Cohen, Professor P Cohen).

2. Control of bacterial gene expression at the level of DNA tertiary structure (Dr D J Lilley, Dr C F Higgins).

3. The formate hydrogen lyase system of E Coli (Dr D G Boxer).

4. Characterisation of proteins of the human neutrophil cell surface using monoclonal antibodies (Dr M Kerr).

5. Mitochondrial bioenergetics, mechanisms of theremogenesis in brown fat regulation of calcium transport in synaptosomes (Dr D G Nicholls).

Applications indicating area of interest and containing the names and addresses of three referees should be sent to Professor P Cohen, Dept of Biochemistry, The University, Dundee DD1 4HN, Scotland, as soon as possible.

(2127)F

STUDENTSHIPS

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF MEDICINE

RESEARCH STUDENTSHIPS

Applications are invited from candidates who have or expect to get a good honours degree in Biochemistry, Pharmacology or a related discipline for studentships, funded for 3 years, which should lead to a PhD The field of study would be concerned with blood platelet behaviour and its relevance to vascular disease.

CV's should be sent to either Professor J R A Mitchell or Dr S Hepinstall at the Department of Medicine, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH. (2091)F

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF MICROBIOLOGY

MRC RESEARCH STUDENTSHIP

The work will combine the techniques of molecular genetics and monoclonal antibodies in a study of a chosen human infection. Candidates should hold or expect to obtain a First or Upper Second Class degree in Biochemistry, Microbiology or other relevant subject. Those interested are invited to contact Professor P J Watt on Southampton (0703) 777222 ext. 893.

Applications giving a full curriculum vitae and the names and addresses of 2 referees should be sent to Professor P J Watt, Department of Microbiology, Southampton General Hospital, Hants. SO9 4XY. Please quote Ref: N (2092)F

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

SERC STUDENTSHIP

Applications are invited for a Research Studentship, tenable from 1 October 1984, from those who have, or expect to have, at least 2:1 Honours Degree in Biochemistry or other appropriate discipline. The studentship could be held in one of the following areas of research.

1. The Redox Chemistry and 3-D Arrangement of Electron Components in bacterial membranes.

2. The regulation of eucaryotic gene expression in fungi and higher plants using biochemical and genetic approaches.

3. The mechanism of action of protein and enzymes important in disease processes including neurochemical studies.

4. The molecular biology of adenovirus.

Written applications with full cv the names and addresses of two rees should be sent within two weeks to Dr D Thirkell, Department of Biochemistry and Microbiology, North Street, St Andrews, KY16 9AL tel: 0334 76161 ext 291) to whom further enquiries may be addressed. (2138)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF MICROBIOLOGY

TWO RESEARCH STUDENTSHIPS

Applications are invited from candidates who hold, or expect to obtain a First Class or 2i Honours degree for the following studentships:

1. An SERC CASE studentship for three years to work on the relationship between the progress of O-acetylation of Staphylococcus aureus peptidoglycan and β-lactam antibiotics. The student will be based in Liverpool and the academic supervisor will be Professor H R Perkins. A single period of at least three months will be spent working in the Glaxo Greenford Laboratories during the three year course. In addition the student will visit Glaxo not less than twice a year.

2. An SERC studentship for three years to work on one of the following areas: encystment in Azotobacter vinelandii; exoenzyme production by Myxobacteria; physiology of thermophilic bacteria.

Applications together with the names of two academic referees should be sent to The Registrar, The University, PO Box 147, Liverpool, L69 3BX. Quote Ref: RV/525/N. (2117)F

OXFORD UNIVERSITY RESEARCH LABORATORY FOR ARCHAEOLOGY

Carbon-14 dating using a 2 MV tandem accelerator, thermoluminescence dating, archaeomagnetism: studentship(s) for DPhil research, suitable for physicists, chemists, earth scientists, anticipating a good honours degree.

Details: Dr M Aitken or Dr R Hedges, 6 Keble Road, Oxford OX1 3QJ, telephone 55211. (2082)F

THE UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY

SERC-CASE STUDENTSHIP

Applications are invited for a studentship to study the regulation of plant gene expression by cell wall fragments. The project will involve both structural analysis of bio-active oligosaccharides released from cell walls and a study of the mechanisms through which their release causes alterations in gene expression. The student will be co-supervised by Dr D J Bowles at Leeds and Dr R Selvendran at The AFRC Food Research Institute in Norwich and it is anticipated that the research will involve extended periods of practical work in both institutes.

Applicants should have, or shortly expect to obtain a first or upper second class Honours degree in Biochemistry, Plant Biology or Chemistry.

Informal enquiries may be made to Dr Dianna Bowles (Tel. (0532) 431751 ext. 7506) to whom applications with a curriculum vitae and names and addresses of two referees should be sent. (2087)F

UNIVERSITY OF LONDON
The Medical College of St Bartholomew's Hospital

SERC RESEARCH STUDENTSHIP IN MOLECULAR ENZYMOLOGY

This Studentship is tenable for 3 years from 1st October, 1984 for work on conformational mobility in Enzyme catalytic sites.

Applicants should have, or expect to obtain this summer, a first or upper second class honours degree in Biochemistry or Chemistry and should have an interest in Enzyme reaction mechanism studied by protein modification, stopped-flow kinetics, isotope inventory studies and spectroscopic techniques. Curriculum vitae and the names of two referees should be sent to Dr K Brocklehurst, Department of Biochemistry, Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, EC1M 6BQ, as soon as possible. (2140)F

UNIVERSITY OF BIRMINGHAM

CASE STUDENTSHIP

'The role of the autonomic nervous system in the control of the elasmobranch gut'.

Jointly supervised by Professor J.Z. Young (M.B.A. Plymouth) and Dr. E.W. Taylor.

Applicants must obtain a 1st or 2(i) degree in Zoology or Physiology.

Full c.v. with 2 referees urgently to Dr. E.W. Taylor, Department of Zoology, The University of Birmingham, Birmingham B15 2TT (2079)F

UNIVERSITY OF READING

DEPARTMENT OF PURE & APPLIED ZOOLOGY

NERC (CASE) STUDENTSHIP WITH INSTITUTE OF OCEANOGRAPHIC SCIENCES (IOS)

Quantitative ecology and associated inorganic chemistry of deep benthic Foraminifera.

A three year studentship to study the distribution of deep-sea benthic Foraminifera in the North Atlantic both locally within the sediment and more widely at different bathymetric depths. Shipbased field work will be carried out in conjunction with the IOS where the subsequent faunal analysis will be carried out. Scanning and transmission microscopy and X-ray microanalysis of specimens will be carried out at the University of Reading.

Applicants (who should hold, or expect to gain, an upper second or first class degree in a suitable Biological discipline) should send a cv including the names of 2 referees to Dr A R Jones, Department of Pure & Applied Zoology, University of Reading, Whiteknights, Reading, RG6 2AJ. (2097)F

UNIVERSITY OF ABERDEEN

DEPARTMENT OF PATHOLOGY

SERC CASE STUDENTSHIP

Applications are invited from candidates expecting to obtain or having a first or upper second class honours degree in basic science with a strong immunological component for a SERC CASE STUDENTSHIP leading to a PhD for 3 years from October, 1984. The successful candidate will study some fundmental aspects of the interrelationship between the coagulation system and cell-mediated immune reactions that are of importance in organ graft rejection and rheumatoid arthritis. The work will also involve evaluation of the effects of selected anti-inflammatory agents in disease processes.

The student will be based in Aberdeen but will spend some weeks each year at the Ciba-Geigy Research Centre, Horsham, West Sussex (Dr M K Jasani). Further details may be obtained from the academic supervisor, Dr A W Thomson, Department of Pathology, University of Aberdeen, Medical School, Foresterhill, Aberdeen AB9 2ZD (0224) 681818 extn 2851 to whom a curriculum vitae with the names of two referees should be sent by 14 June 1984. (2077)F

SEMINARS and SYMPOSIA

Topic and Schedule Change

COLD SPRING HARBOR LABORATORY

The **POLYPEPTIDE HORMONES*** meeting, formerly scheduled for August 28 through September 1, 1984 (Organizers J. Feramisco, O. Rosen, R. Ross) is NOW

GROWTH FACTORS AND TRANSFORMATION

September 19-23, 1984

Organizers:
James Feramisco, *Cold Spring Harbor Laboratory*
Brad Ozanne, *University of Texas Health Science Center*
Chuck Stiles, *Dana Farber Cancer Institute*

There will be sessions on Phosphorylation, Oncogenes and Growth Factor Receptor Systems (EGF, TGF, PDGF, lymphokine, and insulin).

* *A **Polypeptide Hormones** Meeting will be held in 1985.*

For registration information please contact:
Meetings Coordinator
Cold Spring Harbor Laboratory
P.O. Box 100
Cold Spring Harbor,
New York 11724

(NW544)M

WORKSHOP

EMBO INTERNATIONAL WORKSHOP

The Application of Molecular Biology to the Nervous System

LADY MARGARET HALL, OXFORD **23-25 September 1984**

ORGANISERS: E.A. Barnard, M.R. Hanley, J.F. Jackson
Department of Biochemistry, Imperial College, London SW7 2AZ, UK

TOPICS: Molecular genetic and complementary biochemical and immunological techniques applied to receptors, ion channels, neurotransmitters, neural structural components, and nervous system development

SPEAKERS:
M. Ballivet *(Geneva)*
E. Barnard *(London)*
H. Betz *(Heidelberg)*
J. Brockes *(London)*
J. Changeux *(Paris)*
D. Chikaraishi *(Boston)*
R. Craig *(London)*
C. Gorman *(London)*
R. Greenspan *(Princeton)*
C. Henderson *(Geneva)*
E. Kandel *(New York)*
U. Littauer *(Rehovot)*
J. Mallet *(Paris)*
M. Raff *(London)*
H. Soreq *(Rehovot)*
A. Strosberg *(Paris)*
H. Thoenen *(Martinsried)*
A. Ullrich *(San Francisco)*
C. Venter *(Buffalo)*

The meeting will be a residential workshop limited to 65 participants. Topics will be reviewed by invited speakers and will provide the basis for general discussions. Applications should be sent, with a short statements of recent research, to Professor E.A. Barnard (marking the envelope "EMBO Workshop") before 1 July 1984. A number of places are reserved for postdoctoral workers and limited funds are available for support. The registration fee is £100, payable on acceptance, and will provide full board and accommodation for four nights, conference dinner, and reception.

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Applicants should have a First or Second Class Honours degree in Biochemistry or a cognate subject, or be medically qualified. Application for the Academic Year October 1984-July 1985 should be addressed to the Secretary, Course in Neurochemistry, Department of Biochemistry, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF. England. (2086)C

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The fee for the 30 week course is £500. Numbers will be restricted to a maximum of 25 participants. Some assisted places where all or part of the course fees are waived are available to suitable applicants whose fees are not paid by their employer (e.g. persons working in Universities or similar institutions and holding non-tenured posts). Further details and application forms may be obtained from: Dr I C Shaw, Laboratory of Toxicology and Pharmacokinetics, University College London, 5 [illegible] versity Street, London WC1E 6 England. 01-388 2411 ext 276.

Closing date for applications — July 1984. (2144)C

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Further details: D J Langley, Microbiology, University of Surrey, Guildford, GU2 5XH. (2119)M

LECTURES

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LUNTEREN, THE NETHERLANDS, 2-5 Oct. incl. 1984

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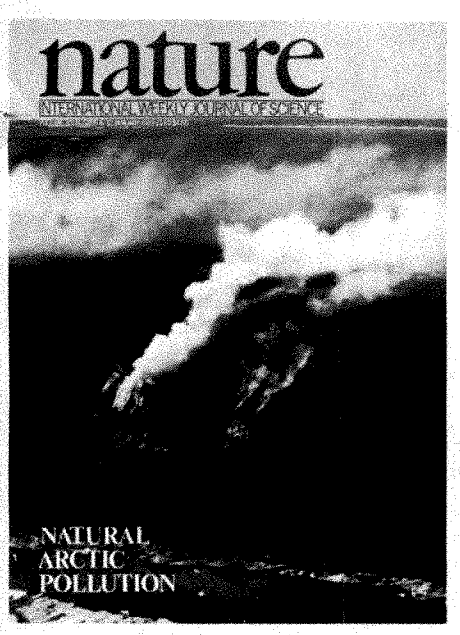
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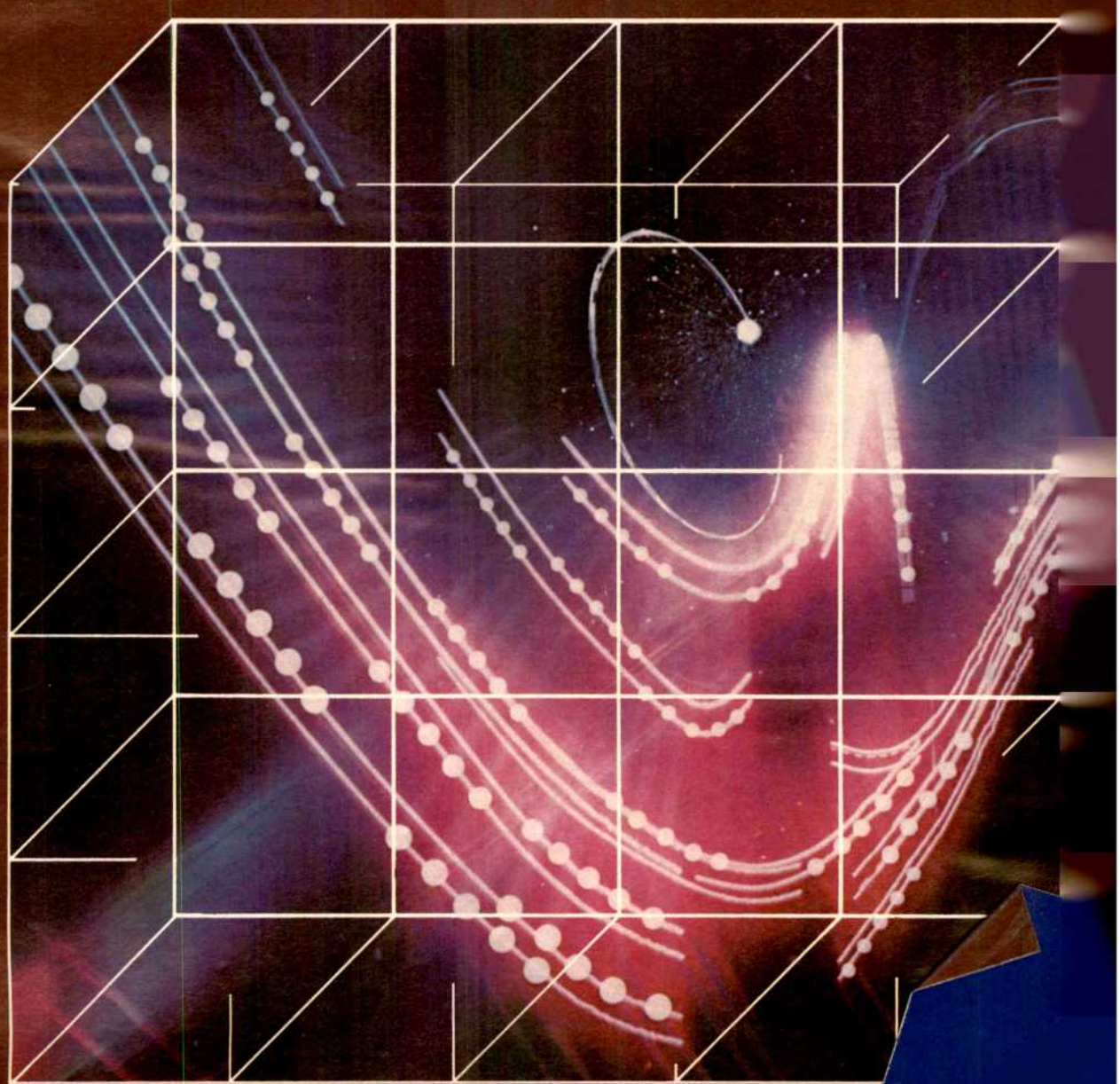
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COVER



Underwater photograph of the giant clam *Tridacna maxima*. Several thousand eyes lie along the margin of the fleshy lobes or syphons which extend beyond the edge of the animal's shell for maximum exposure to light. The study on page 446 describes the visual system of *Tridacna* and shows that some retinal cells contain receptors that are sensitive to ultraviolet light. (Photo: Dr H.G. de Couet).

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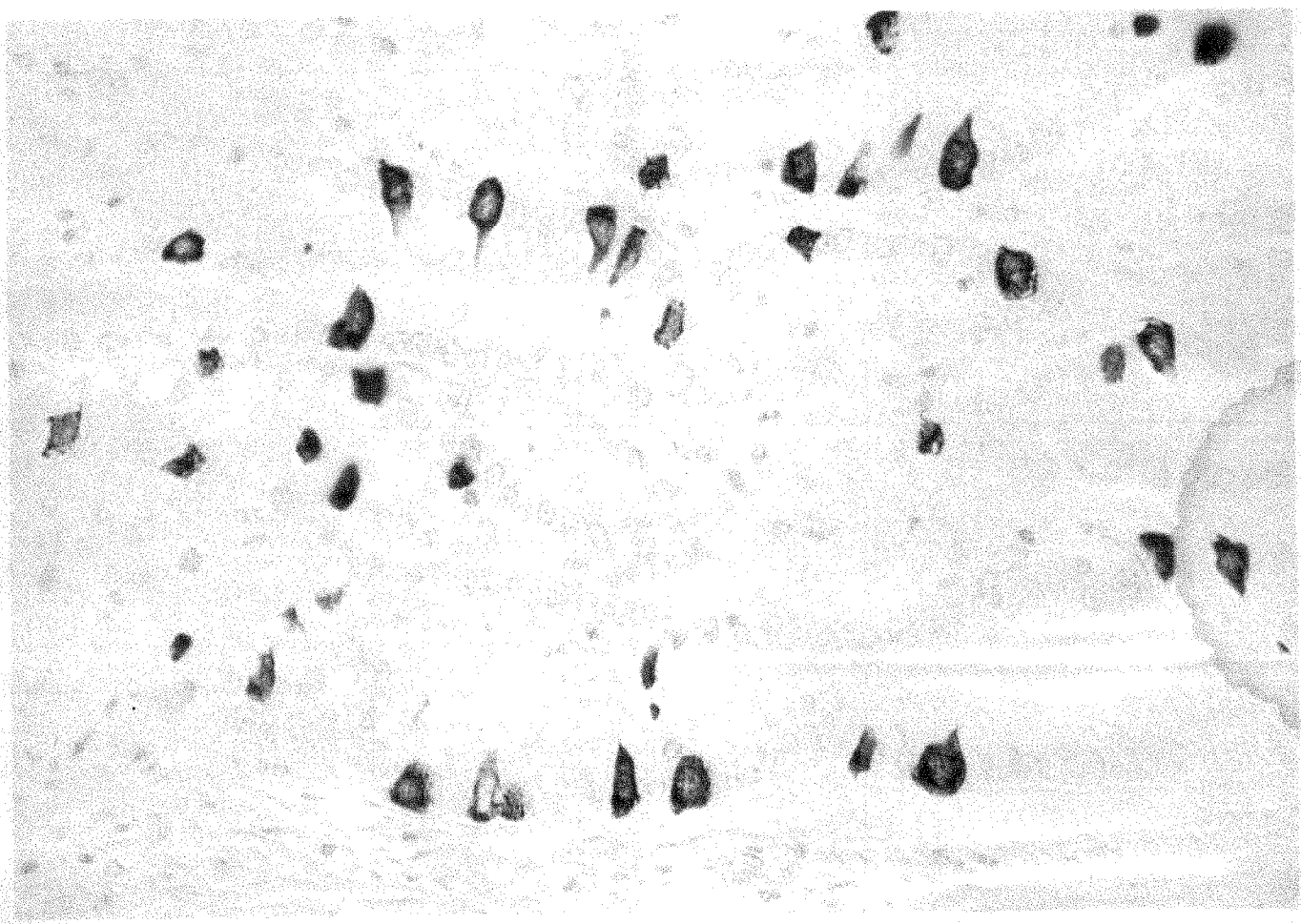
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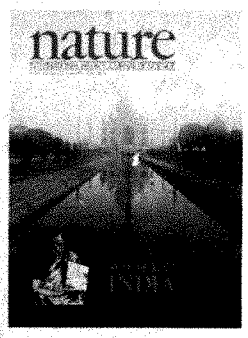
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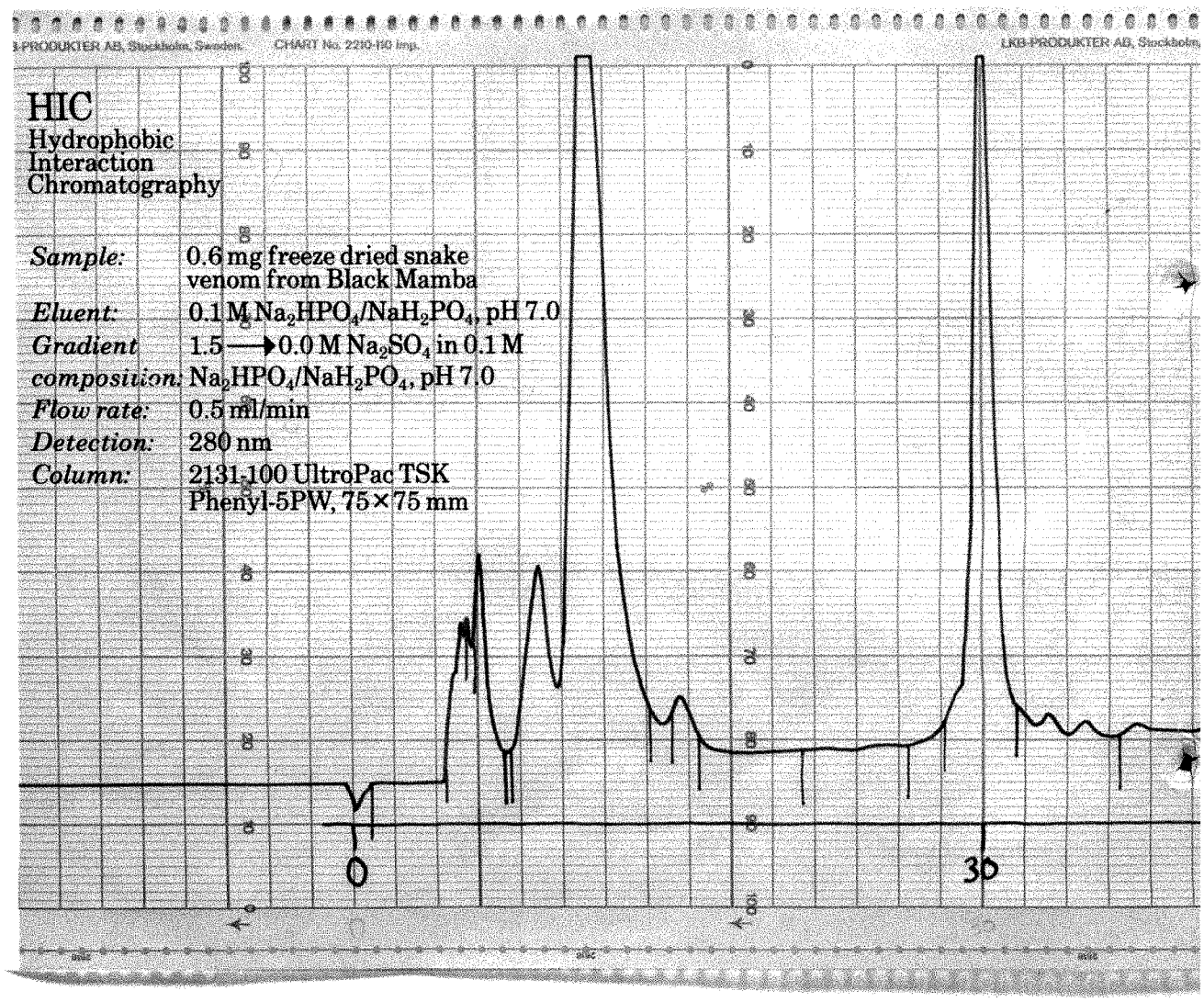
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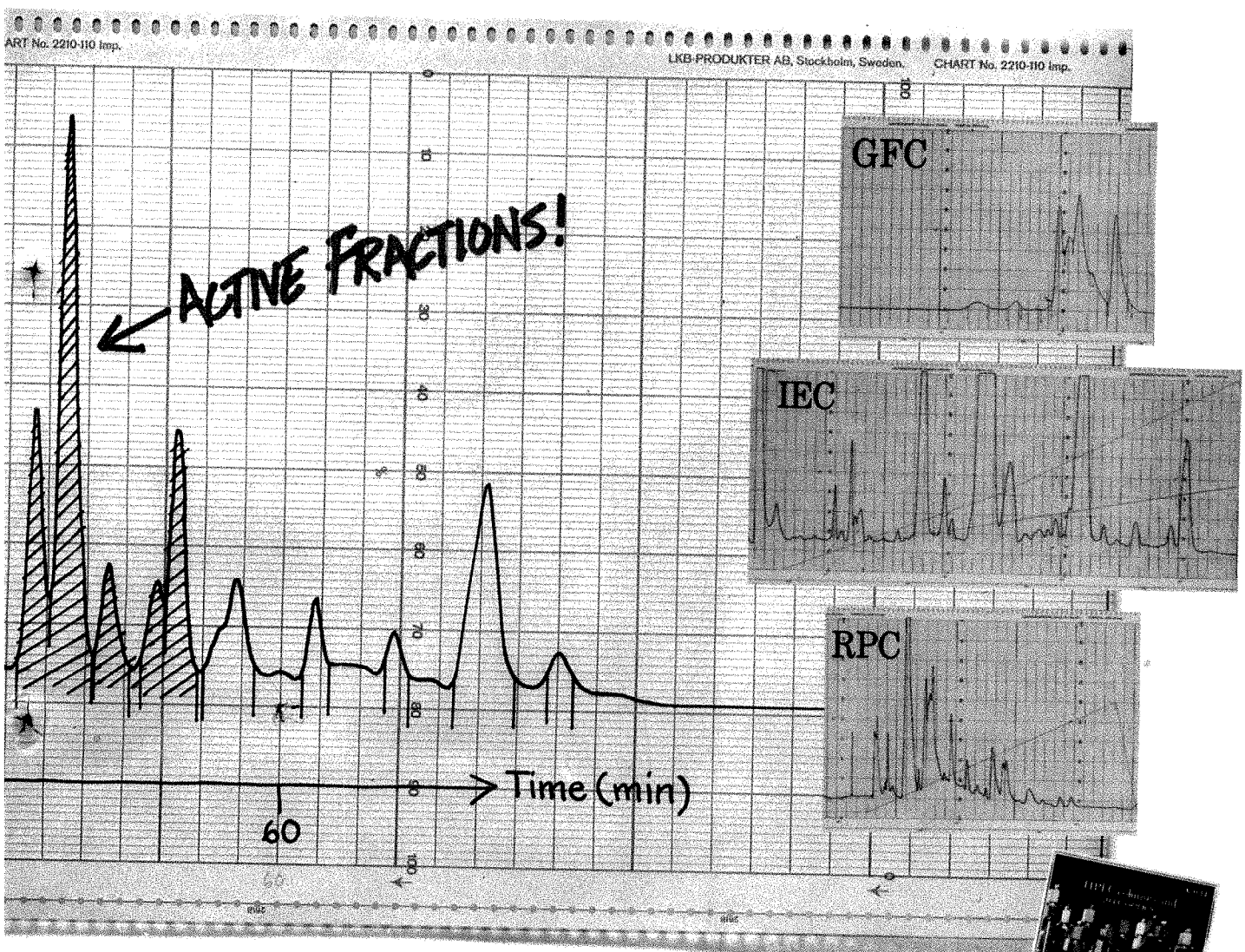
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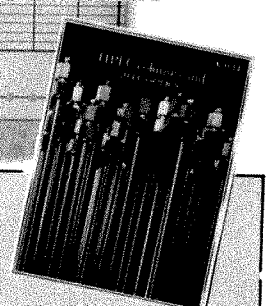
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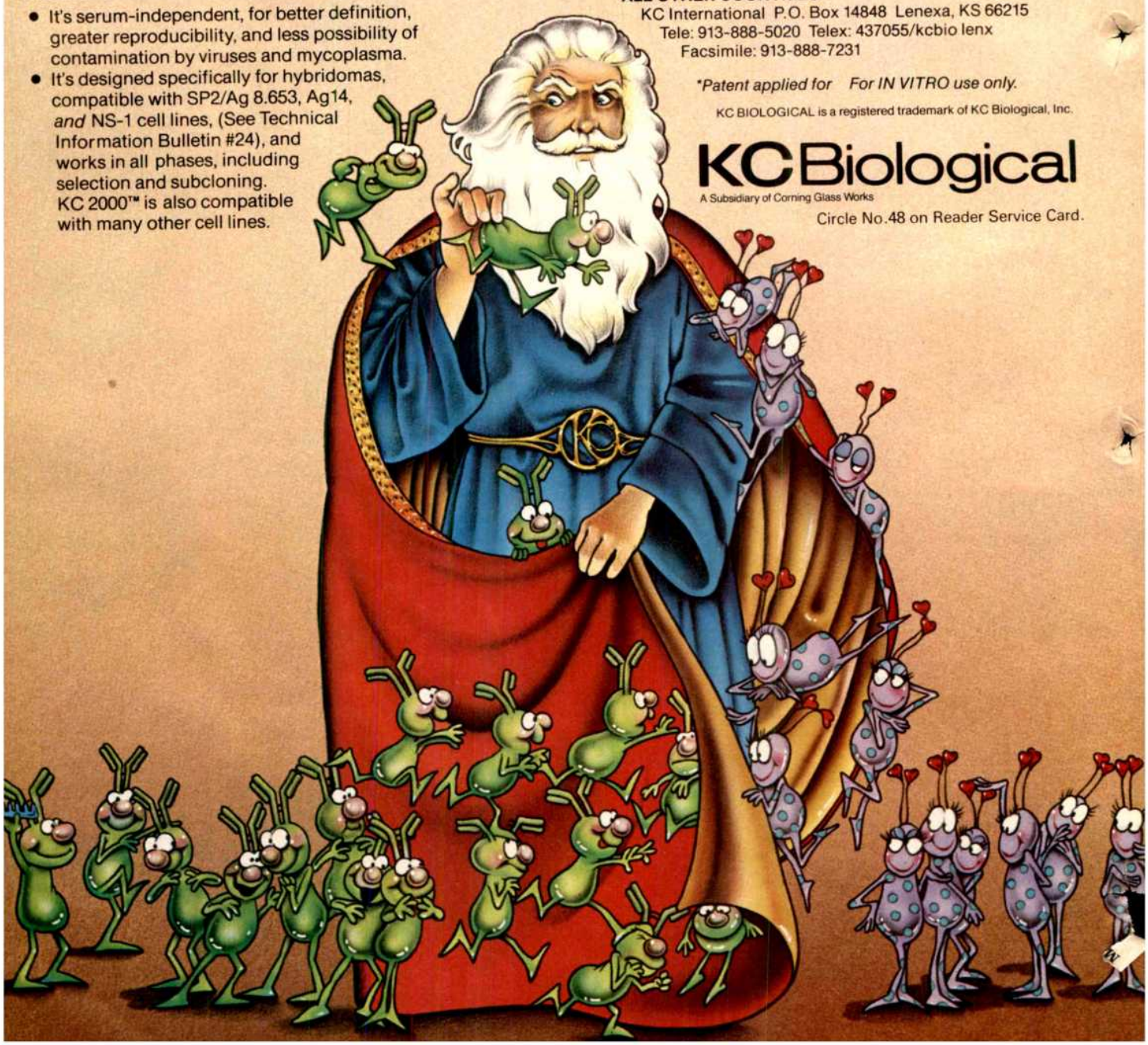
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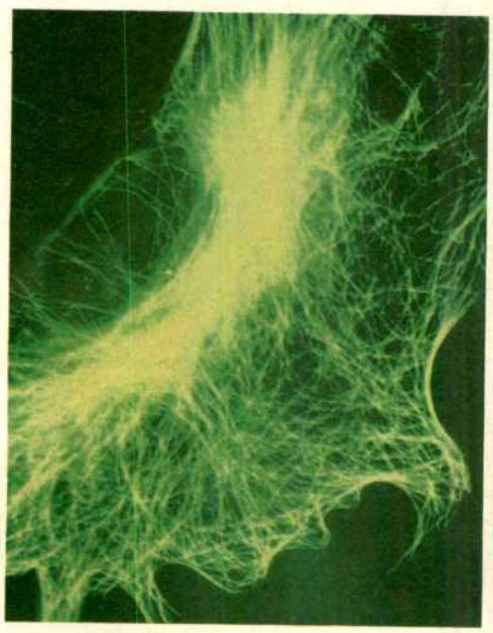
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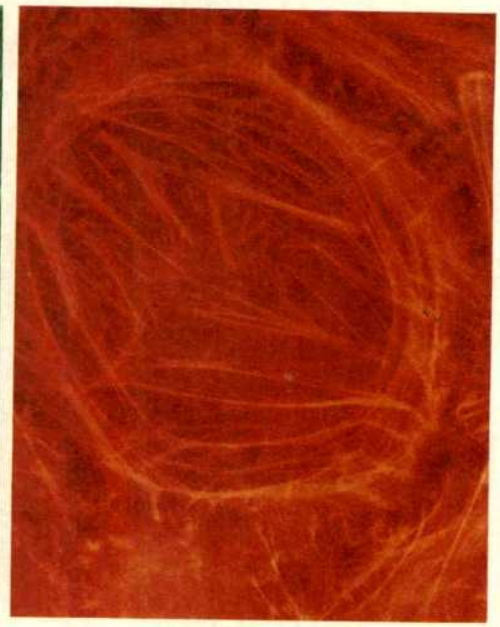
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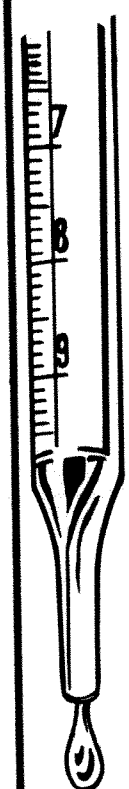
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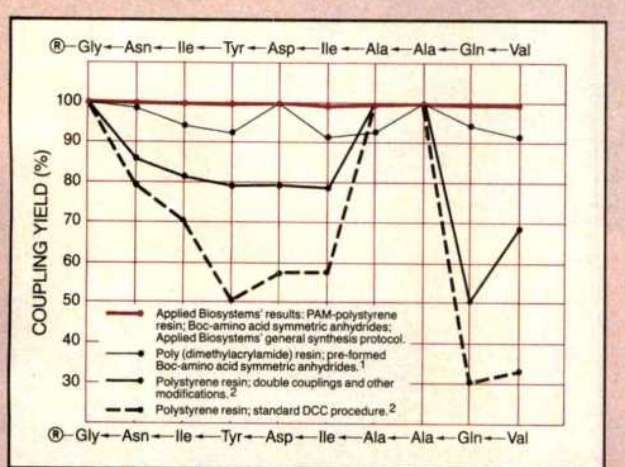
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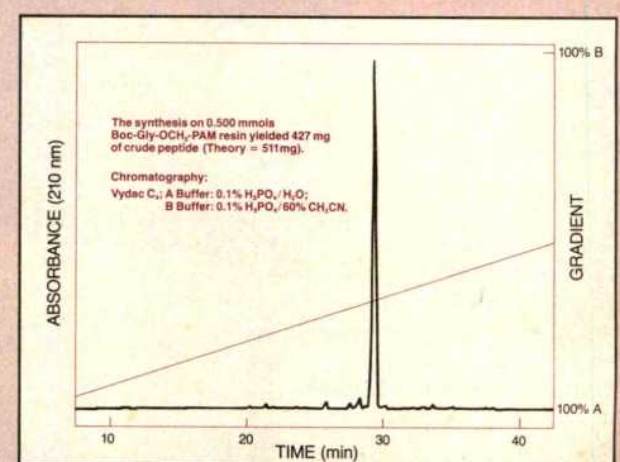
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The new Model 430A Peptide Synthesizer was introduced at FASEB and Analytica, and will be exhibited at the ASBC Meeting. For more information, circle No. 08



Amino acid incorporation during assembly of Acyl Carrier Protein residues 65-74.



HPLC chromatogram of crude, HF cleaved Acyl Carrier Protein (65-74).

ANALYTICAL METHOD	STEP YIELD (%)									
Quantitative Ninhydrin Monitoring[3].	—	99.9	99.6	99.5	99.4	99.1	99.2	99.2	99.1	98.9
Preview Quantitation by Solid Phase Sequencing of Protected, Resin Bound Peptide[4].	—	—	99.4	—	99.3	99.1	99.2	—	98.9	98.7
	RELATIVE AMINO ACID EQUIVALENTS									
Amino Acid Analysis of HF Cleaved, Deprotected Peptide	1.00	0.97	0.90	0.94	0.97	0.90	0.96	0.96	0.94	0.98
Amino Acid Residue	Gly	Asn	Ile	Tyr	Asp	Ile	Ala	Ala	Gln	Val

Step yield quantitation and amino acid analysis results for Acyl Carrier Protein (65-74) chain assembly using Applied Biosystems' general synthesis protocol. Only single couplings were used throughout the synthesis (except for Gln).

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1. Reza Arshady, Eric Atherton, Derek Clive, and Robert C. Sheppard, *J. Chem. Soc. Perkin Trans* 1, (1981) 529–537
2. W.S. Hancock, D.J. Prescott, P.R. Vagelos, and G.R. Marshall, *J. Org. Chem. 38* (1973) 774
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4. Stephen B.H. Kent, Mark Rieman, Mary LeDoux and R.B. Merrifield, *Proc. Int'l. Conference: Methods of Protein Sequence Analysis*, 1982

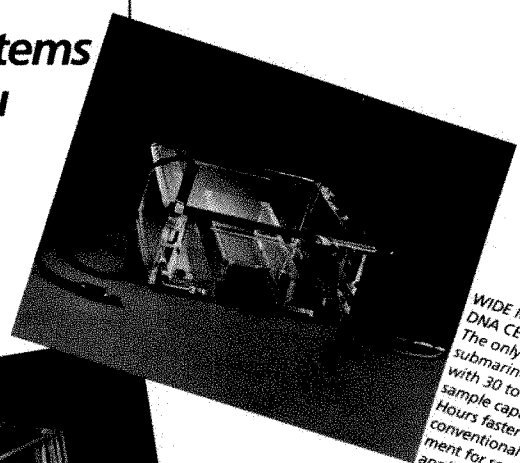
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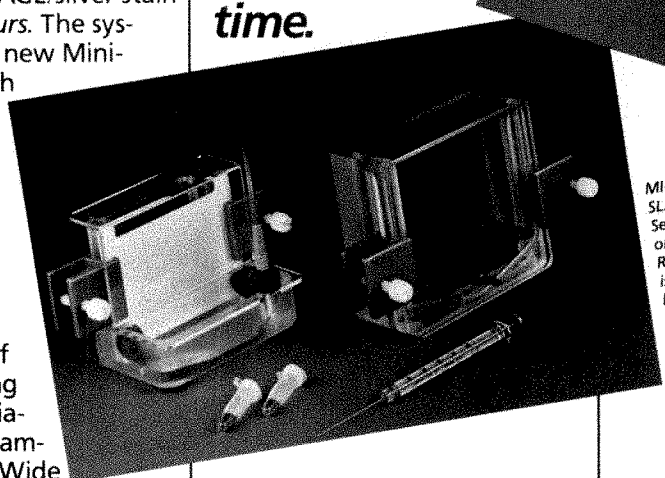
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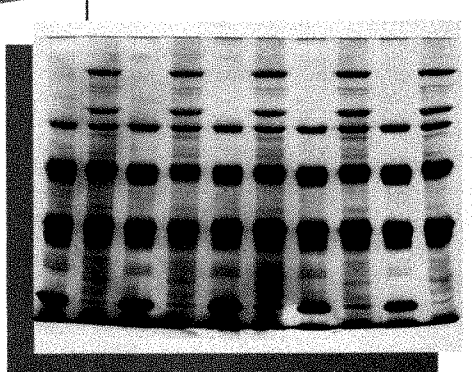
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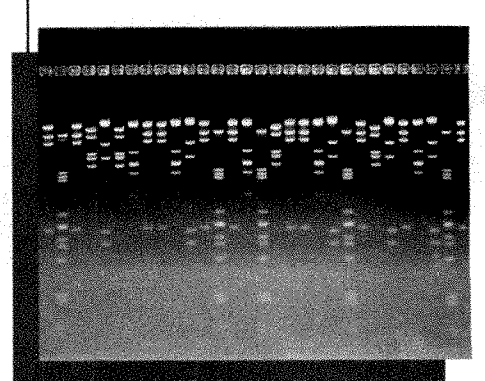
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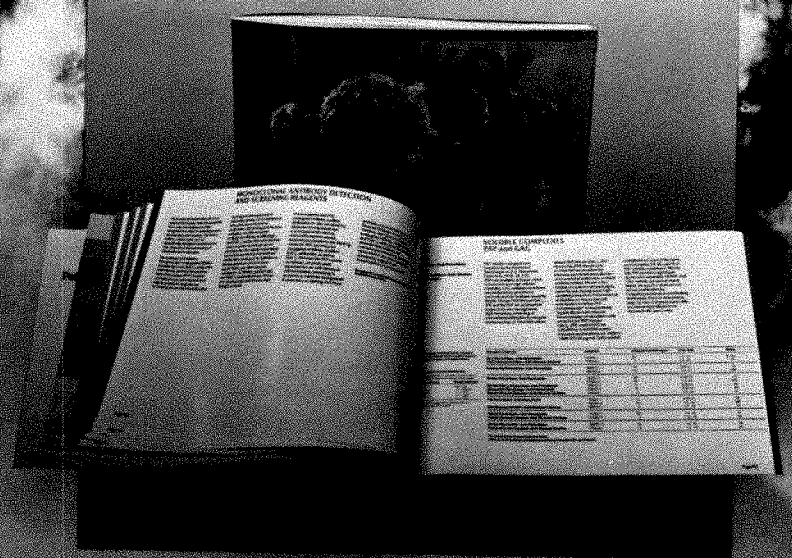
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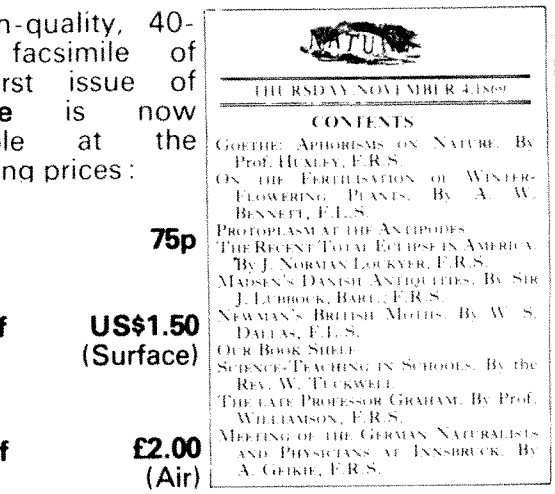
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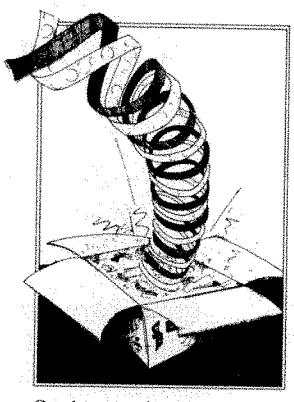
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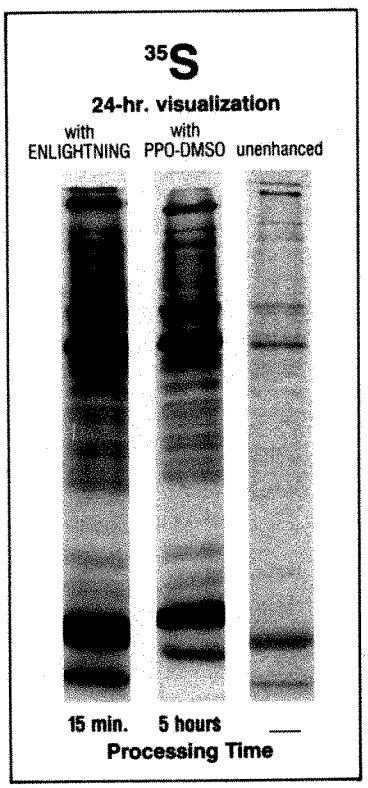
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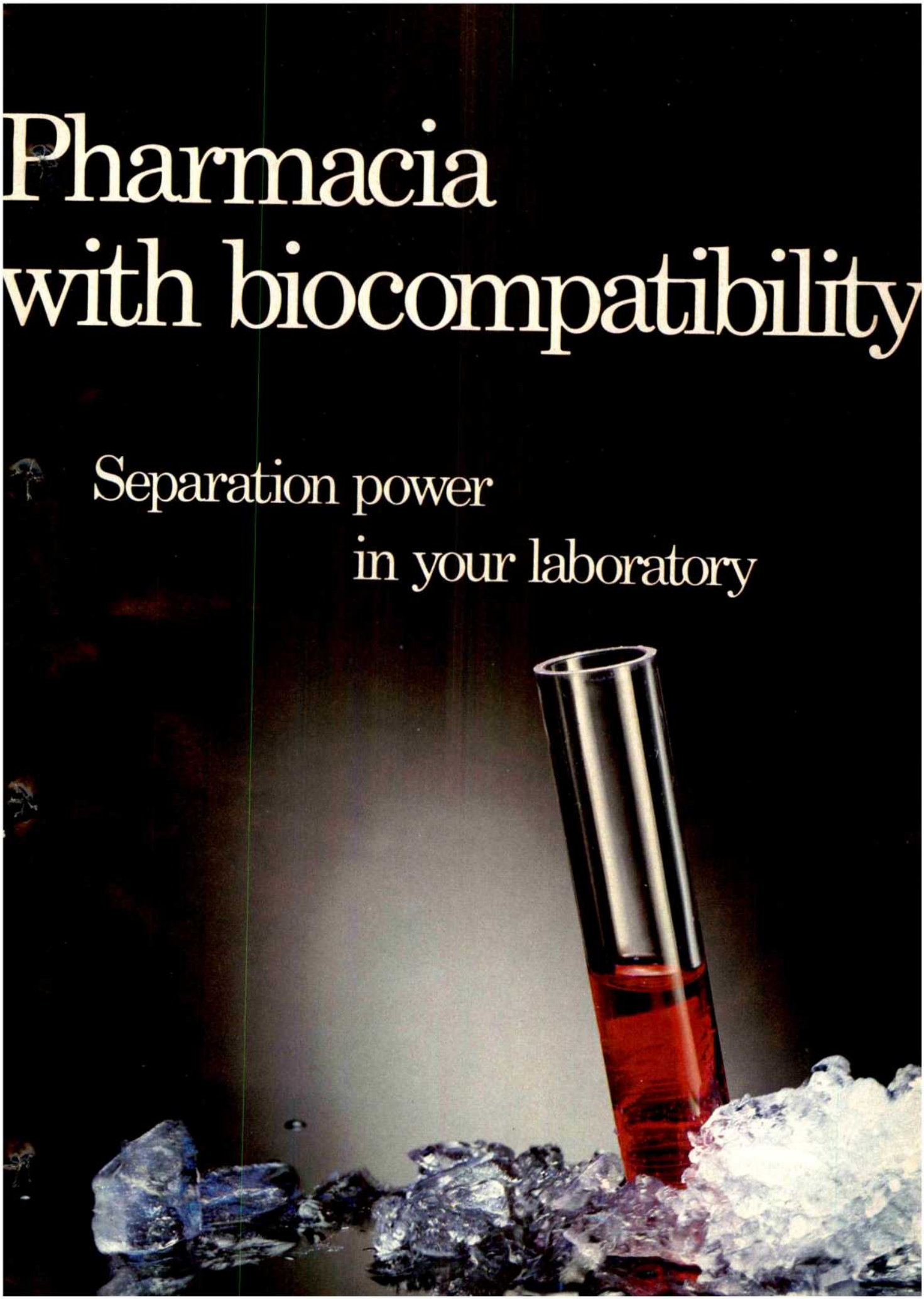
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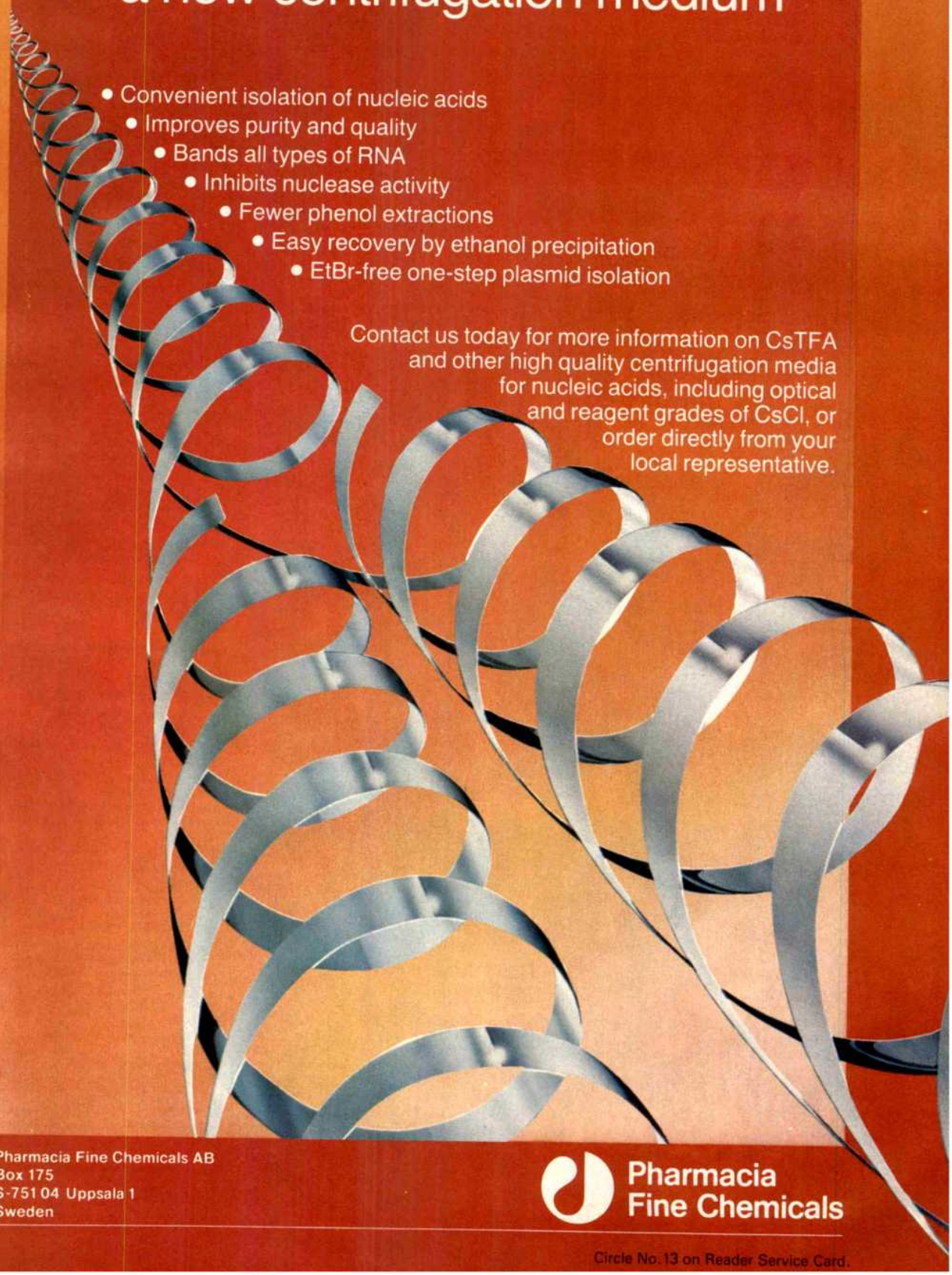
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Handling the human embryo

Proposals for the organization of British practice are a useful curtain-raiser for the government's own report. But physicians should regulate themselves before the government steps in.

THE Council for Science and Society has usefully stolen a march on the British Government by publishing (last week) the report of a study of new techniques of conception, some old (such as artificial insemination), some futuristic (such as cloning). The document (*Human Procreation*, OUP, £3.95) has been put together by a committee under Dr G.R. Dunstan, an academic Anglican with at least fifteen years' experience of these problems, but with a good sprinkling of hard and level-headed biologists to help tell one end of a blastocyst from another. The document appears some three months before the report of the official committee under Mrs Mary Warnock is due to report. Being a liberal and even modestly adventurous tract — Dunstan would allow the use of fertilized but unimplanted embryos for research, is neutral on surrogate motherhood (saying that it should neither be encouraged nor made unlawful) and sternly dismissive on the choice of the gender of unborn children. In all probability, this is where the Warnock committee will stand. Certainly that committee's recommendations on the legal framework for embryonic manipulation cannot be less liberal without seeming unhelpful.

The Dunstan document is said to have been designed to be thought-provoking, which mostly it is. But on some issues the group seems to have decided that a dash of pragmatism is the only sensible way of avoiding arguments that can never be settled. Thus the committee dodges the question of when life can be said to have "begun" by argument by analogy with the law on abortion in the United Kingdom. Sperms and ova are alive, so "life" must mean something else, but development is continuous after fertilization so why not extend the limiting age of a fetus for legal abortions to other practices now possible for the first time, the study of development in viable embryos, for example? The trouble, as the Dunstan committee must know, is that there is a substantial body of opinion in Britain opposed to that limit, and eager to see the Abortion Act repealed. If the Warnock committee makes recommendations along the same lines (which it should), there is certain to be a revival of agitation against the rules on abortion.

Privacy is dealt with more robustly. The issue arises in connection with existing practices such as artificial insemination (estimated at 4,000 cases a year, not much greater than in the early 1970s) but, more generally, whenever a child is born with genes not all derived from its social parents. On the question whether such a child should at some stage be told something of the source of the genes, the Dunstan committee is for "openness within the family", which would imply that a child would eventually have a right to know as much about, say, the donor of semen as the parents themselves were told at the outset. But the committee would protect the confidentiality of the donor, largely because it fears that the supply of donors would otherwise dry up but also because it holds that a child's relationship with its social parents should not be prejudiced by the intervention of a third party, either a semen donor or a surrogate mother. The view is consistent. The obvious difficulty is that responsibility for keeping genetic records of these shadowy but essential third parties will rest with physicians whose practices are already shadowy enough. But especially in Britain, where the Data Protection Bill will do very little to strengthen the privacy of individuals in relation to the state, it would be too dangerous to ask for a centralized data bank of the genetic parents of other people's children. Is there a case for physicians' professional associations shouldering this task?

The committee's weakest conclusion is that it would not be permissible to allow parents to choose the gender of their children. Technically, this can already be done by amniocentesis and abortion, but an unwelcome gender is not a legal ground for abortion. Soon, easier ways of doing this job will be available. The starting point for much of Dunstan's argument is that, if stable couples are prevented by some disability from procreating naturally, they should be helped by whatever techniques are available to produce children in some other way. Why draw the line at gender-rigging? A farming family of a dozen daughters and no sons may be as seriously handicapped as one that is infertile. So why not acknowledge that physicians should be encouraged to offer even such a service if there are strong grounds for believing that the results would be socially beneficial?

With its pragmatic view of the problems of embryo manipulaton, the Dunstan committee inevitably is less concerned with the legal than the administrative changes of the framework in which the physicians deal with their patients. (But it would change the law to protect donors of sperm from paternity suits.) Writing for a British audience, it asks that the National Health Service should take responsibility for providing whatever services are available, that donors (of anything) should not be paid for their services and that there should be more provision for counselling than at present. The committee looks enviously across the English Channel at the centralized service by means of which the French health services arrange for sperm donors to be married couples with at least one healthy child. The committee acknowledges that there are problems with surrogate mothers (who would not act as hostesses without reward of any kind) but seriously underestimates the difficulties of ensuring that all services like these can somehow be swept up into the public sector. That is why there can in the long run be no escape from a system in which individual practitioners offering services as different as artificial insemination and sex determination should be registered (even licensed) by their professional associations and should contribute data about their practice to some confidential data bank run by the same association. That, it is to be hoped, is what Warnock will say. □

Liberation language

THE introduction to the Dunstan report by Professor John Ziman, who quotes from Dante's *Purgatorio* a definition of life as

. . .one
quick, sentient soul, of its own self-aware,

includes the following explanation of a now-unfashionable useage:

The report is written in English, a language which has a common gender for statements applying to masculine and feminine, male and female, without distinction. Sometimes the grammatical form of the common gender and masculine coincide, but that is no impediment to its use. We have employed this grammatical facility because it yields a prose less cluttered and more elegant, and as readable. □

Small beer, no skittles

The British research system needs to spend more on research grants, less on machines.

UNWITTINGLY, the British Science and Engineering Research Council (SERC) has given a telling explanation of why the United Kingdom is considering withdrawal from the European high-energy physics laboratory, CERN, at Geneva. The council's science board, the chief source of research grants for British academics whose interests are not bound up with space, astronomy and high-energy physics, last week published a statement of what it calls its strategy for "core science" — what is left when the great machine-created baronies have been catered for (see *Nature* **309**, 297; 1984).

The results are shocking. The board spends just over £70 million a year, which is divided into three roughly equal parts, one of which goes to keep the bodies and souls of graduate students and doctoral fellows in some loose correspondence, another on the running of central facilities (such as synchrotron and laser sources) and a third, roughly £25 million a year, on research grants to working scientists in all disciplines. Having proudly calculated that 12,000 people are dependent on it for research money, it omits the simple arithmetical calculation that there is less than £2,000 a year for each person on its books.

Lopsidedness also leaps out from this eight-page glossy production. University research in physics, for example, costs the board between £5 and £6 million a year. This is hardly the kind of cash flow on which a strategy could be founded. The board says that it is having to refuse nearly a third of grant applications that deserve to succeed. Maybe it should be worrying instead that, with such tiny sums of money at its disposal, it is not much more seriously overwhelmed.

Balkanization

To be fair to all concerned, these sums are not the whole of what the British research councils spend on the support of academic research. The specialized spending boards into which SERC is balkanized also make grants to academics but, of necessity, the successful applicants there are almost always those whose projects dovetail neatly with the planned use of some machine, observatory, Earth satellite or the like. Other research councils chip in as well; the Medical Research Council spends more on academic biological research than does the science board by a factor of two; the Agricultural and Food Research Council, more deeply committed to its own institutes, also helps, as does even the Natural Environment Research Council. The result, however, is a conspicuous lopsidedness. Why are physics and chemistry research, core sciences if there are any, supported on such a miserly scale?

The usual answer is that that is how the pattern of spending has developed, and that the supply of research grants cannot be too different from the pattern of demand, because the mechanism is self-correcting. This complacent argument overlooks the simple truth that if you starve a field of funds, and spend the best part of a decade telling academic scientists that they would be better employed in other fields, in information technology or engineering for example, they will eventually go elsewhere, intellectually or geographically.

Core science

In Britain, the problems of core science have been multiplied by the perverse pursuit of over-rational stratagems for economizing in resources. High-powered lasers are in fashion? Then the managers of the research enterprise quickly calculate that they could not provide every likely group with up-to-date equipment so let there be a single central facility that everybody can use, well designed (and slowly built). This is probably the most effective device yet discovered for turning honest scientists into committee-people, skilled above all at winning allocations of time at facilities which are always more elaborate than would have been necessary for a single purpose — and whose lifetime is assured for years and even decades to come. So it turns out that with the passage of time, young men who embark with enthusiasm on working as attendants of the big machines (even the small big machines) discover that they are no longer practitioners of the craft that attracted them but rather managers, negotiators, sometimes even diplomats.

Nobody suggests that it would be possible to turn back the clock so far that the practice of science in the physical sciences would be exclusively modelled on what happened in the nineteenth century. It is however likely, with the present distribution of resources in Britain between machine science and small-scale science, that the pendulum has swung far too far towards the machines. There is no evidence that people dislike working with the large machines but that is not the point. What matters is that the way in which the machines claim people's allegiance for years on end ensures that the community of scientists in a country as small as Britain is less flexible than it might be, less able to seize new opportunities.

The science board seems at least to have understood the way in which changes should be brought about. Its strategy document says that it needs more money and that if it had more money it would give first priority to research grants and the last to the strengthening of machine facilities. But how long does the science board think it would take to effect a noticeable change in the pattern of scientific research if on the one hand it is going to adjust its distribution of resources only when the total is increasing and if at the same time it follows its own prescription of giving particular attention to projects which have industrial potential? One of the constantly repeated mistakes in the management of British civil science in the past few decades is that with each new economic crisis, the research councils whose responsibility is to ensure the health of basic science have felt it necessary to demonstrate that they too have to put their shoulders to the wheel, helping with chemical engineering one decade, information technology the next.

Mercifully, there are signs that even this preoccupation may soon be modified. In the past six months, SERC, recognizing the dangers of over-dependence on machines, has tried to create a set of circumstances in which the two big spenders among its committees, the astronomy, space and radio board and the nuclear physics board, would be merged, presumably so that they would then take a more sceptical view of what their enforced partners were supporting. It is a well-known failing of even the best intentioned systems for distributing money for research that peer review is harder to trust as the scale of projects grows. Unfortunately, SERC has for the time being defeated its chairman's plan to bring about this marriage. He (or his successor) should not however let the matter rest there. The case for having rational means of distinguishing between the merits of different proposals is too strong to be forgotten.

The grant-making establishment needs also to safeguard itself against repetition of the mistakes that have recently caused such trouble. Logical though it is to think of serving many users and saving money at the same time by the provision of central facilities which all experimenters can use, the system is also a recipe for diseconomy. Would it not be better, as in the United States, that if a person thinks it would be valuable to have a machine for accelerating particles, or producing intense monochromatic beams of light, or even perhaps a machine for producing neutrinos, he should not be prevented from applying for the money he needs by the argument that the project is too big for one man to handle? Of course that is literally true. But elsewhere, in the United States particularly, there is now a long record of successful exploitation of the principle that the most enthusiastic user of a novel kind of machine is likely to be the man who gave the reasons why it should be built. And then, of course, if two people have the same idea for a new machine at the same time, there can be a competition. Either way, the enterprise is likely to be carried out more economically than would otherwise be possible, fewer people will acquire a vested interest in its perpetual continuation and, indeed, there would be many occasions when designers of new experiments on a large scale were treading on their own heels, anxious to get rid of what they had been anxious to build only a little while earlier. □

American Association for the Advancement of Science — New York

Administration hints at clampdown on biotechnology exports

AMID charges from the Department of Defense (DoD) and US intelligence agencies that the Soviet Union is applying biotechnology to biological warfare research, the Reagan Administration is beginning to suggest that biotechnology may have to be brought under export control regulations designed to halt the flow of "militarily critical technologies" to potential adversaries.

The Reagan Administration's interpretation of the export control rules has been the subject of controversy, particularly in the crackdown on exports of computer components and in DoD's recent efforts to shut out foreign nationals — even those from NATO countries — from scientific meetings held under its sponsorship.

At the AAAS meeting here, John Birkner of the Defense Intelligence Agency predicted that "dual use" biotechnology — items with both peaceful and military applications — would sooner or later have to be restricted. He asked scientists in industry and at universities to help DoD learn "how our technology may be turned against us" so that a "prudent" list of militarily critical technologies can be compiled for biotechnology.

Items listed as militarily critical can be exported only with a licence issued by the Department of Commerce. To qualify for the list, a technology must not already be possessed by "principal adversaries" nor available to them from a third party. Birkner said that specialized polymers, synthetic elastomers and detectors for chemical and biological warfare agents would all be likely candidates for inclusion on the list.

The charges that the Soviet Union is already exploiting biotechnology for biological warfare have emerged in recent weeks from official DoD sources, the Central Intelligence Agency and a series of articles in the *Wall Street Journal*. DoD's recent report on Soviet military power asserts that the Soviets have an active research and development programme to investigate the utility of biological weapons; Birkner said that "there are at least seven biological warfare centres in the Soviet Union that are under strict military control" and which are applying "selected aspects of genetic engineering to their work". The *Wall Street Journal* articles made similar accusations, and in turn have been sharply criticized by many US researchers for imputing sinister motives to research activities that are indistinguishable from US efforts in biotechnology and basic genetic engineering.

The putative activities of the Soviet Union would constitute a violation of the 1975 convention banning the possession or development of biological weapons. The United States had earlier accused the Soviets of violating the treaty (and the 1925 chemical warfare treaty) by using toxin and chemical weapons in Afghanistan and South-East Asia.

Meanwhile, some new light has been shed on the US military's own programme of recombinant DNA and hybridoma research. Thomas Dashiell of the Office of the Secretary of Defense appeared at the meeting here with a list of 43 biotechnology research projects funded by DoD as of 30 April. The bulk of the projects deal with development of vaccines against disease endemic in areas where US troops might be sent or against possible biological warfare agents, such as anthrax. Other projects are aimed at developing enzymes to decontaminate chemical warfare agents and at producing materials such as epoxies, lubricant stabilizers, biofuels and marine antifouling agents.

The research, which is divided between in-house efforts (mainly at Fort Detrick in Frederick, Maryland — the former biological warfare research establishment) and sponsored research at universities (among them Massachusetts Institute of Technology, Texas A&M University, Rockefeller University, Purdue University and the Universities of California, Massachusetts, and Maryland), is all unclassified. Dashiell suggested, however, that if the research moves beyond the basic stage — which he says is not likely to happen for eight years at least — some of the development work may be subject to secrecy restrictions.

Stephen Budiansky

Peter David adds: In Washington meanwhile, Congress received an unexpected signal last week that the Reagan Administration may be softening its stance on the control and publication of research findings. Dr Edith Martin, Deputy Under-Secretary of Defense for research and engineering, announced during a hearing of the House Science and Technology Committee that the administration intended to rely on formal classification procedures to keep sensitive research out of the hands of foreign nations.

Apparently reading from the still-unpublished report of a senior interagency group examining technology transfer, Dr Martin said it was the policy of the administration that "no restrictions may be placed on the conduct or reporting of research that has not received national security classification".

Although the statement referred specifically to "fundamental" research, it was taken by observers at the AAAS meeting to imply that the Department of Defense (DoD) had dropped a controversial plan to restrict the publication of applied research that was sensitive but not formally classified. Drafted as part of a wide-ranging review of the Pentagon's approach to international technology transfer, the plan would have insisted that DoD contracts for work that was both "applied" and "sensitive" should in future give the Pentagon the right to prevent publication of the resulting findings.

In meetings with universities in recent months, Dr Martin had argued that total reliance on classification, in which it would be necessary to show that publication of the research would constitute a direct threat to national security, was too inflexible. But her proposal to create a new category of sensitive research whose publication would be controlled by DoD was fiercely opposed. Three universities — Stanford and the California and Massachusetts Institutes of Technology — wrote to DoD and to the White House science office claiming it would prevent them from undertaking certain categories of research for DoD. □

Europe uneasy at CERN rival

DR Herwig Schopper, director general of the European Organization for Nuclear Research (CERN), is sowing seeds of dis sent to the ambitious US plan for a massive new particle accelerator. Speaking here at the AAAS meeting, Schopper suggested that the tunnel now being completed for LEP, CERN's 100-GeV electron positron collider, could be used for a future proton machine with capabilities approaching those of the US design, and at substantially lower cost.

While careful to stress the importance of "complementarity" between European and US efforts in high-energy physics, Schopper clearly implied that the United States could find itself competing with CERN for the claim to the next large accelerator.

The United States made an initial commitment to its proton collider, known formally as the Superconducting Super Collider (SSC) (and earlier known as the "Desertron"), last year when the High Energy Physics Advisory Panel endorsed the concept. The panel, which represents the particle physics research community and which makes recommendations to the Department of Energy (DoE), at the same time voted to abandon Brookhaven ▶

National Laboratory's unfinished ISABELLE accelerator, recognizing the unprecedented concentration of technical and financial support that SSC would require.

As now envisaged, SSC would attain centre-of-mass energies in the neighbourhood of 40 TeV — sufficient, in the view of current theory, to explore the so-called Higgs sector. Leon Lederman, director of Fermilab, who also spoke here, said that experimental exploration of this energy region is vital to resolving the "crisis" that Lederman says his "theoretical colleagues" now find themselves in. "They're speculating wildly", he says, for lack of solid data.

According to cost estimates developed earlier this year at a marathon session at Lawrence Berkeley Laboratory (some 150 researchers from the national laboratories and universities were, as one participant put it, locked in a room by Department of Energy officials until they could agree on some definite numbers to be used in preparing next year's budget request), SSC could be built using existing magnet technologies for $3,000 million, with a completion date in 1994. DoE is now considering whether to submit a request for research and development funds in the budget for fiscal year 1986. Lederman says that it would probably take two or three years to develop a definite design that optimizes costs. The "reference design" document produced at Lawrence Berkeley considered three different combinations of ring size and magnet field strength, and concluded that costs would be roughly eqaual in each case. The original idea of SSC, and thus the name Desertron, was to use relatively low field, "cheap" magnets in an enormous ring that could presumably fit nowhere but the southwestern desert.

Lederman said he considered the $3,000 million figure to be a maximum, as it assumes the use of existing "dumb" magnet technology, as he put it.

Schopper, on the other hand, placed a price tag of $500 million on the European version of the proton collider, the cost savings coming from the use of CERN's existing tunnel and injectors. The energy of the machine, however, would be only 10 to 12 TeV if existing magnets technology is assumed, although it could be as much as 18 TeV if the as yet undeveloped Nb3n 10-tesla magnets become available in time.

Both Lederman and Schopper spoke of the need to coordinate US and European plans; both also said they had little idea how this could actually be accomplished. Lederman said, however, that it would probably be a political impossibility to persuade the countries in question to build both machines. He also questioned whether the CERN proton collider's energies would be sufficient to explore the Higgs sector.

Schopper, in the face of repeated questions about the relative cost-effectiveness of the two plans, said "It's no use starting a fight now"; more work needs to be done on the technology before a decision can be made, he said. And, apparently referring to hints of some new and puzzling discoveries coming out of CERN's SPS collider, suggested that in the two to three years needed for technology development, "the physics might be different".

Schopper suggested that another possible add-on to the LEP tunnel — one that certainly would not compete with SSC — would be an electron–proton collider.

Meanwhile, the United States is already worrying about selling the most expensive basic research project yet to Congress. Presidential science adviser George Keyworth was recently in Japan trying to drum up some tangible support from the Japanese Government for the project. And DoE officials are only half facetious when they suggest that the SSC ring might be arranged to pass through at least three states to boost regional support in Congress.

Stephen Budiansky

Intellectual property

Consider the monoclonals

BRITISH universities seem to be rebelling against the mounting pressure on them to become more responsive to commercial considerations. The Committee of Vice-chancellors and Principals (CVCP), in a preliminary response to a government discussion paper on intellectual property rights, rejects the idea that universities should seek to protect research inventions with patents wherever possible, citing as an example the "serious disadvantages for generations of investigators" if monoclonal antibodies had been rigorously protected. And in a formal reply to a plea by the Engineering Council to spend more of their grant on training engineers, the committee reaffirms its opposition to having grants specially earmarked for engineering and its distaste for a rapid increase in provision for science and technology at the expense of other subjects.

CVCP dismisses the government's "green paper" on intellectual property, published at the end of last year, as "somewhat superficial". The paper made some radical suggestions on how the Patent Office might be moved out of the civil service and suggested some changes to intellectual property law. But, say vice-chancellors, it pays too little regard to the difficult balance that university researchers must maintain between possibilities for commercial exploitation and their commitment to intellectual progress. And CVCP dismisses the "implied criticism" that academics exchange information too freely and take protection too rarely. Universities have, the committee says, a clear policy on patenting. The government should, before considering legislation, thoroughly examine all aspects of intellectual property, including such problem areas as copyright and computer software. Some are concerned that biotechnology, in particular, might be adversely affected if very broad patents impede research.

Beyond this CVCP does not go, thus perhaps inviting the criticism that it, too, is being somewhat superficial. The committee excuses itself by blaming an unrealistic timetable for comment imposed by the Department of Education and Science. Discussion has had to be brief and consultation has been less than complete. A fuller response will be prepared in due course.

The committee has however managed to find the time to prepare a full response to the Engineering Council, which earlier this year accused universities of not using their grant in the way that was intended. The council announced that it wanted a ten per cent swing in student numbers from arts-based to science-based subjects within the decade. That objective is thought by vice-chancellors to be totally unrealistic, given the pressures that already exist on the university system, and many vice-chancellors feel the Engineering Council has weakened its case by overstating it.

Tim Beardsley

Argentinians safe

Washington

THE new democratic government in Argentina is wasting no time in trying to re-establish contacts with expatriate Argentinian scientists and to offer assurances that the personal and political vendettas of past regimes will not be repeated.

Dr Carlos Abeledo, under-secretary for science promotion and director of the national research council of Argentina, was in the United States last week to spread the word among the hundreds of Argentinian scientists here that if they want to return they will be welcome and there will be financial support for them. Abeledo said that he did not expect more than a few dozen to take up the offer this year, but that he was also hoping to involve the expatriate scientists in the country's scientific effort through such activities as reviewing for Argentinian scientific journals, reviewing grant applications, and taking in Argentinian research fellows.

Abeledo was due to meet with some 200 Argentinian scientists at the American Association for the Advancement of Science meeting in new York; the previous week he was present at a gathering of 100 Argentinian scientists now living in France.

Albeledo was among the hundred or so scientists sacked in 1976–77 for political — and sometimes personal — reasons by the government. One of the first acts of the new government this year was to recognize the right of those scientists to return to their positions.

Stephen Budiansky

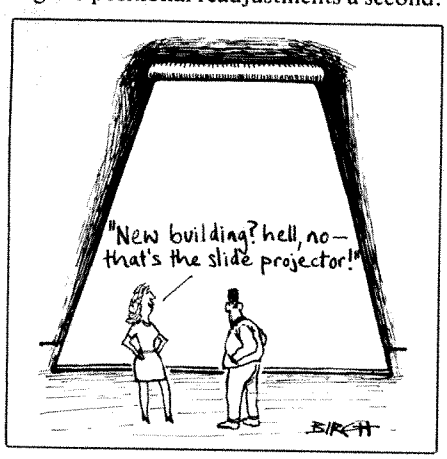
US universities

Benefactions abound

Washington

UNIVERSITIES in the United States are enjoying a spectacularly profitable spring this year. In April alone, the University of Texas at Austin announced that an $8 million donation by an anonymous Texan would enable it to endow 32 new chairs in science and engineering, and the University of California said that it had received the biggest single private gift in its history: $36 million for a new telescope. There may be more to come. According to a new survey, universities and colleges are becoming America's favourite philanthropy: voluntary contributions to higher education reached a staggering $5,000 million in 1982–83, an increase of 6 per cent over the previous year.

The University of California gift comes in the form of cash, property and art masterpieces bequeathed by Mrs Marion Hoffman, widow of the car dealer, Max Hoffman. The money is to form part of a $100 million fund with which the university intends to build a 10-metre telescope of novel design at Mauna Kea on Hawaii by the end of the decade. It will consist of 36 six-foot hexagonal mirrors arrayed to form a continuous optical surface. The mirrors will be kept aligned to a precise optical figure by a system of computer-controlled sensors and actuators capable of performing 100 positional readjustments a second.



It will have four times the light-gathering power of the venerable Hale Telescope on Mount Palomar.

A bonanza of similar proportions will befall the University of Texas as a result of the $8 million donation by a local industrialist. The $8 million is to be matched by five Texan foundations, and another $16 million will come from the state-funded Permanent University Fund. Together, the package will enable the university to establish 32 chairs endowed for $1 million each. The university expects filling the chairs to take several years. It will be recruiting in chemistry, physics, mathematics, molecular biology, computer engineering, microelectronics, computer-assisted design and manufacturing and materials engineering.

The universities of California and Texas are traditionally among the biggest recipients of private and corporate philanthropy. A survey now published by the Council for Financial Aid to Education reports that, in 1982–83, voluntary donations to the University of Texas exceeded $107 million, while the University of California received more than $135 million. In proportion to their size, however, neither matches Harvard and Stanford, which received donations of more than $126 million and $91 million respectively.

Where is all this money coming from? Increasingly, it seems, from private business. Corporate philanthropy has at last outstripped donations from foundations and reached more than $1,000 million in 1982–83, 14 per cent more than in the previous year. That means that the lion's share of donations comes from four distinct sources: corporations, foundations, alumni and private individuals. Each gives about $1,000 million in a year, while religious denominations give an estimated $200 million in total.

Nearly a third ($1,500 million) of all the money contributed is in the form of unrestricted gifts. Some $300 million is earmarked for academic salaries and $750 million for research. But the gifts are distributed extremely unevenly. Major private universities receive an average of $21 million each, about five times as much as their public counterparts. And ten universities accounted for a full $690 million of the gifts in 1982–83. Harvard and Stanford lead the pack. Eight other institutions, receiving between $50 million and $60 million each, are: Minnesota, Columbia, Cornell, Massachusetts Institute of Technology, Yale, Princeton, Southern California and Pennsylvania.

Peter David

Genetic engineering

Picking up the pieces

Washington

PROPOSALS from private companies for the field-testing of recombinant organisms will still be considered by the Recombinant DNA Advisory Committee (RAC) of the US National Institutes of Health (NIH), despite the cloud created by a federal district court ruling in favour of anti-genetic-engineering activist Jeremy Rifkin (see *Nature* 24 May, p.296). That ruling has temporarily halted Dr Steven Lindow's experiment on ice-nucleating bacteria and barred RAC from approving any other experiments involving NIH funds that would deliberately release recombinant DNA into the environment.

But the ruling specifically exempted private companies, which are not legally bound by RAC decisions, although they have been complying on a voluntary basis. The reasoning was that if RAC was not allowed to consider proposals from private companies, the companies could simply proceed without RAC's approval.

Although a final decision has not been made by NIH, RAC staff said last week that as far as they knew two deliberate release proposals from private companies were still on the agenda for the next RAC meeting, on 1 June. Advanced Genetic Sciences Inc. (AGS) is seeking approval for a field trial virtually identical to Lindow's, and Cetus Madison is submitting a proposal for the field testing of plants with genetically-engineered disease resistance. RAC will hear all of the Cetus Madison proposal and part of the AGS proposal in closed session, in accordance with the standard procedures for the safeguarding of trade secrets.

In a letter to the executive director of RAC dated 18 May, Rifkin requested a moratorium on consideration of all deliberate-release proposals and said he was appealing the district court's decision on that point. Rifkin's attorney, however, said they would not appeal until RAC had decided definitely on how to handle the June meeting.

Meanwhile, the University of California has filed an emergency appeal against the temporary injunction against Lindow's experiment. According to Lindow, the experiment must begin by around 25 May or be postponed until autumn. William Anderson, attorney for the university, said that the court failed to examine RAC's deliberations on the Lindow proposal, which, he said, met the legal requirements of a "hard look" at environmental effects. The appeal also argues that Rifkin failed to exhaust available administrative remedies before filing suit, as he failed to file comments or attend either of the RAC meetings at which the Lindow proposal was discussed. Rifkin says he did not know about those meetings and was out of town when they took place; announcements of the meetings and requests for public comment were, however, published in the *Federal Register*.

Rifkin's attorney, Edward Lee Rogers, said that he thought neither argument would stand up; the *Federal Register* announcement was "cryptic", he said, and did not make clear that this was the first deliberate-release proposal; and he said the "equivalency" defence — that RAC in fact carried out the equivalent of a legally-required environmental assessment — is available only to agencies whose principal responsibility — and thus expertise — is with environmental issues.

Stephen Budiansky

Venture capital

Stealing universities' thunder?

A NOVEL Anglo-US joint venture launched this week aims to sponsor the commercial development of research projects carried out in British universities. The two partners in the new venture, to be called the Research Corporation Trust, are Investors in Industry (3i) and the Research Corporation, of New York. The trust will, through a subsidiary, evaluate, patent and license inventions throughout their lifetimes and will use the proceeds to sponsor more university-based research.

The trust is likely to prove controversial. In return for its free advisory and patenting service to universities the trust will normally expect resulting patents to be made over to it. The obvious question is whether the fruits of research supported by the British taxpayer might not be enjoyed overseas. Mr Ivan Momtchiloff, a general manager of 3i and a director of Research Corporation Trust, said recently that British inventions would be exploited by the trust in Britain "where humanly possible", unless to do so was "obviously silly". A slightly different line has been taken by Dr John P. Schaefer, president of Research Corporation, who says the trust "will not only help strengthen science and technology in the United Kingdom but also lead to a mutually beneficial exchange of technology between the United States and Europe."

The two partners in the new trust are not obvious bedfellows. Research Corporation in the United States is incorporated under not-for-profit law, and the new trust in Britain will, it is hoped, receive charitable status. The Investors in Industry group is, however, an established large industrial investment group, backed by major British banks, whose goal is undeniably to make a profit. Mr Momtchiloff says that 3i will profit from any companies that it acquires or spawns to develop the inventions originated by the trust, and from licence income.

A major spur to the formation of the new trust has been the recent abolition of the first-refusal right on much publicly-funded British research previously enjoyed by the National Research Development Corporation (now part of the British Technology Group). The 3i group has previously formed companies based on university research but the process has often been protracted and difficult. The group was, according to Momtchiloff, considering a new approach based on research grants when it learnt that Research Corporation in the United States was thinking of setting up a British operation; a partnership was the natural result. The corporation already has the special skills and extensive experience of working with university departments to enable a similar scheme to be set up in Britain. The trust will also benefit from Research Corporation's established patenting and legal support services.

The initial capital in the new scheme is suprisingly modest — £1 million from each of the two partners, with an additional £50,000 each to start the ball of research grants and license income rolling. Many of the initial grants will be for only a few thousand pounds — to buy a specific item of equipment for instance. The trust will seek mainly to interest young researchers who may have more difficulty than their elders in attracting research funds.

The trust's share of royalty income, net of expenditure, will be returned from the subsidiary operating company to be distributed as more research grants. In the United States the arrangement between universities and the new trust's parent company is that the institutions take 60 per cent of royalties and the trust 40 per cent. Arrangements between institutions and their employees are more variable, and the opportunity to take an equity stake in a new company would depend on the individual circumstances. **Tim Beardsley**

A UK venture

A VENTURE capital fund launched last week in Britain will provide £15 million over 4 years to invest in new and emerging private companies working in high technology. The Charterhouse Japhet Venture Fund has already raised this sum and has made its first investments. Its preferred areas are telecommunications, microelectronics, health care and biosciences.

The capital that the new fund has available puts it immediately in the ranks of the larger venture funds in Britain. The investors are prominent UK pension funds. The investment directors are Dr John Walker, who comes from the Investors in Industry group, and Mr Ron Sheldon. Walker emphasizes that his approach extends to providing active management support and marketing experience.

Most of the investments will be of more than £250,000, but some "seed capital" for new product development will also be available. The £15 million will spread over 20 to 25 major investments, of which the fund will be the lead investor in about a half. Walker is critical of other venture capital funds that attempt to gain control over a young company by taking a majority investment. He swears that the new Charterhouse fund will always take a minority stake so that control remains with the originator of the company, a principle Walker considers extremely important.

Walker has a significant personal stake in the new fund. He declines to reveal the amount but admits that he "probably won't have to worry about cash" if the fund succeeds. He and Sheldon, by demonstrating their personal commitment, will inspire company management to confidence, he believes. Tim Beardsley

Agriculture

Old crop ripe for revival

Waalre, The Netherlands

ONE of the first plants to be exploited by man in South-East Asia and Oceania could once again become a major commercial crop in the region if the efforts of Professor Michiel Flach of the Agricultural University at Wageningen bear fruit.

The crop is the sago palm, natural stands of which cover some 2 million hectares in Indonesia, Papua New Guinea and the Philippines. These areas are underexploited, says Professor Flach, in a region where it would be prohibitively expensive to plant other crops. There are also 200,000 hectares of planted sago stands, mostly in Malaysia. There is a small but growing market for sago starch, not only for food use and industry (textiles and paper) but also for production of high-fructose syrup.

Professor Flach's espousal of the sago cause began in 1970, and with backing from the World Bank, the Food and Agriculture Organization (FAO) and the Dutch Government he organized international symposia on the subject in 1977 and 1980. Earlier this year there was a discussion on sago at an FAO workshop in Jakarta, Indonesia and Flach is now organizing a third symposium — this time in Japan where there is considerable interest in the crop.

A new sago starch factory is to be constructed in Indonesia, where the cultivated stands can produce at the rate of 2.5 to 5 tonnes per hectare. World starch production from cereals is about 1,360 million tonnes so the 2.2 million hectares of sago palm could produce only some 1.5 per cent of world production, but in some areas the contribution from sago palm could be important. In Sarawak, for instance, the palm is an important crop in peaty soils, and in swampy areas in the tropics sago palm will grow quickly (8 years to maturity compared with 13 years in other areas). In food energy terms the palm will out-yield any other crop.

Professor Flach does not see pure sago starch as a challenge to the central role of rice, but in Indonesia more and more people are eating bread, and sago starch added to bread flour can significantly improve quality. The country imports around a million tonnes of grain a year, and any application of the indigenous sago palm that would help reduce imports would be of vital importance.

Most of the work on sago palm is directed towards production in Indonesia, where the ministry of public works is studying the potential use of coastal swamps in sago growing. And now there is a major project sponsored by the World Bank. "Suddenly nearly everybody is working in the field", says a surprised Professor Flach. **Casper Schuuring**

Software copyright

MITI retreat on "piracy"

Tokyo

JAPAN's Ministry of International Trade and Industry (MITI) has abandoned its controversial plans to make changes in the laws governing software copyright — changes which, in the US view, would have made "worldwide software piracy legal". But plans are going ahead rapidly to strengthen the software industry by setting up a new Y 290 million (£900,000) database research centre and by merging software organizations into a single promotional group.

What worries MITI is that Japan's software industry is not growing sufficiently rapidly to enable it to catch up with the West. In the United States and Europe, the explosive growth of the software industry has been fired by the appearance of huge numbers of small entrepreneurial software houses selling software packages wherever a niche can be found for them. Japan is only just entering this stage. Until now, growth has been hampered by big businesses which want to construct their own software or have its specially built for themselves alone, rather than buying a package off the shelf.

To try to boost the industry, MITI called for a new law "more clearly to protect the rights of computer programmers" and to give programmers the right to "allow or refuse the use of software by a third party". At the moment, software rights are provided in an ill-defined way by copyright laws administered by the Ministry of Education, Culture and Science (MESC)'s Cultural Affairs Agency.

But instead of the fifty years' protection given under Japanese copyright law or the seventy-five years given under US law, MITI proposed that only fifteen years be guaranteed — even though some of the early US software still in use is already about that old. What is more, the new law made it possible to require a software developer to sell his software in exchange for a "reasonable payment", whether or not he wanted to sell it, when the sale was "in the public interest" or when use was refused without a "justifiable reason".

Despite strenuous denials from MITI that the plan had been thought up to help Japanese companies to get access to US software technology, the US software industry reacted with fury. Many also felt that the successful lawsuit brought by IBM against Fujitsu and Hitachi — two of Japan's top computer makers — which forced them to pay huge sums in compensation for allegedly copying and reselling IBM software had stirred MITI into proposing such a radical change in the law.

Joining in the US protest was MESC's Cultural Affairs Agency, which refused under any circumstances to yield its control of software copyright to MITI. The combined opposition was eventually sufficient to overwhelm MITI, but not before a remarkable series of semantic squabbles between MESC and MITI over whether software was akin to literature, and thus clearly material that should be protected by copyright, or an "industrial process" which MITI could claim should be dealt with by its patent department.

While MITI's plans for new software laws have reached a dead end, progress has been made this past week in regrouping Japan's database research and development groups. MITI is to use funds from one of its more profitable sidelines — running the nation's bicycle races — to draw together the fragmented database industry. Under the bicycle race law, MITI supervises the "fair and safe conduct of all bicycle races" and puts the profits into the improvement of "bicycle and mechanical technology" as well as improvements in "medical care and public hygiene". Although funding databases may be stretching the law's original intentions, Y170 million has been earmarked from the fund as an initial payment to help set up a new institute for database research. A further Y120 million is to be provided by a group of 20 industrial companies, including Mitsubishi, Hitachi, Toshiba, NEC and Fujitsu, that MITI is bringing together along with a number of academic researchers in the universities to form the new group.

The aim is to bring database development up to Western levels. MITI was spurred into action by the result of a survey showing that 679 databases were in use in Japan — ranging from economic statistics to chemical literature — but only 157 (23 per cent) of them had been developed inside the country. Major problems have yet to be overcome, however, to bring together the different groups working on databases. Some databases are already being developed in universities and in the Japan Information Centre of Science and Technology with support from the Science and Technology Agency.

A large database is also under construction in MITI's patent office to store patent information. As it covers a very wide range of fields, it is essential that it be properly integrated into the overall strategy. The problem is the familiar one in Japan of getting competing ministries to work together — particularly difficult in this case because part of MITI's aim in setting up the group was to establish that databases belonged in its territory rather than that of the Ministry of Posts and Telecommunications. The problems are expected to be resolved by the summer and the location and structure of the centre to be agreed upon.

A new industrial grouping is also almost ready for those involved in other areas of software development. Both personal and commercial software are now to be grouped together into one umbrella organization under MITI's aegis to provide a centralized group capable of more "organic" activity. The details of the new organization will be released by MITI at the end of the month.

Alun Anderson

Hungarian science

Making the most of society

HUNGARIAN biology must come to terms with the challenge of computers, information technology and systems analysis, Dr Janos Szentagothai, president of the Hungarian Academy of Sciences, told the academy's general assembly last month. The theme of the meeting was the relationship between biology and society — an important subject in a country with a considerable agricultural and food industry and environmental legislation going back to the mid-nineteenth century.

Dr Szentagothai concentrated on three main topics: the academy's role as scientific coordinator in the preparation of socio-economic decisions, basic research of "outstanding future importance" and participation in certain aspects of scientific public life. He stressed the setbacks suffered by Hungarian science as a result of the swingeing cuts in research expenditure two years ago. Even a year's moratorium on spending on new equipment and the servicing of equipment already purchased from abroad had, he said, greatly hindered the utilization of the country's leading intellectual resources.

During the meeting, Deputy Premier Jozsef Marjai announced a special government credit of 200 million forints (£33 million) for research, in addition to what had been originally planned, most of it to be allocated by the academy for basic research. Nevertheless, Dr Lenart Pal, the general secretary of the academy, urged that other ways of generating research funds must be encouraged, such as the formation of "profitable partnerships and subsidiaries" by research institutes and industry.

It was, however, what Dr Szentagothai called "scientific public life" which provided the major innovation this year. Starting in 1985, the academy's elections are to be "democratized", with multiple candidates for each elective post (president, vice-president, presidium members) and tenure normally restricted to two consecutive five-year terms. A 14-person nominating commission, headed by Professor Bruno Straub, was elected by this year's assembly, and will begin work next March on preparing the necessary lists of candidates.

Vera Rich

Creation research and Noah

SIR — Jukes's commentary on his caricature of creation (*Nature* **308**, 398–400; 1984) is a superb illustration of the unscientific approach of the anti-creationists, especially his conjuring up of "data" to support his polemic. Contrary to Jukes's demonstrably false claims, I have never sent graduate students to do field research on fossil human footprints (although I have led them on Grand Canyon field studies), have never even considered specializing in the "ecology of Noah's Ark", and certainly did not (indeed, could not) synthesize the Taylor's sophisticated museum display with which I am pictured. In the "half (or one-tenth) truth" category, Jukes labours the fact that I wrote a children's book on fossils, but mentions neither that the book was written for Christian families (not at all for public schools) nor that I have co-authored two two-model books with quite a different approach for public schools and, with a highly respected biologist, four widely used college programmed textbooks in biology. His misrepresentation of my colleagues and other aspects of creation science are even further off the mark.

If Jukes (unfortunately rather typical of the anti-creationists) cannot be trusted accurately to report simple, easily obtainable facts verifiable in the present, how can anyone trust his wild-eyed speculations about the complex and unobservable past? It is sad to see a man of Jukes's former stature sink so low. But if *Nature* (a refereed journal?) and those evolutionists who are scientists want to retain a claim to fair and objective scientific rationality, they must separate themselves publicly from Jukes's pitiful tantrum. At least Jukes's article will make thinking people everywhere wonder what is wrong with eliminating "the current methods for teaching evolution" if he exemplifies them.

GARY E. PARKER

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● Jukes replies — Professor Parker incorrectly states that I wrote a caricature of "creation". My article was about creationism, a rather different topic.

Parker says he has "never sent graduate students to do field research on human footprints". However, this topic of graduate study is listed in the Institute for Creation Research (ICR) General Catalog (p.14). His "Grand Canyon field studies" would presumably be related to his assertion (Parker, *Dry Bones*, pp.53–54) that the canyon was formed 5,000 years ago by the Great Flood. Although he "did not . . . synthesize the Taylor's sophisticated *sic* museum display", an essentially similar pictorial representation appears in *Dry Bones* p. 24.

Parker's speciality on the ecology of Noah's Ark was inferred by me from *Dry Bones*, which devotes about 16 pages to Noah's Ark and the flood, with pictorial details of the fauna and their food supply.

Parker says I did not mention that *Dry Bones* was written for Christian families. The book is offered for public sale in the ICR book catalogue, where it is described as "fun as well as educational". Despite Parker's assertions, *Dry Bones* has been used for science instruction in at least one public school, where it aroused protest by families that include Christians. I did not find it necessary to publicize his other literary efforts.

I made no misrepresentation of his colleagues; I let them speak for themselves. Parker speaks of my "wild-eyed speculations about the complex and unobservable past". The best response is to quote some of Parker's own pronouncements. For example:

"If the fossils are the plants and animals drowned in Noah's Flood, then they would all be about five to seven thousand years old." (*loc. cit.*, p. 44).

"Before the Flood there were many more plants without seeds compared to seed plants. Plants without seeds didn't survive the Flood as well seed plants."

THOMAS H. JUKES

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Semen and AIDS

SIR — In the letter "Semen and AIDS", by G.M. Shearer and A.S. Rabson (*Nature* 15 March, p.230), some consequences of the physiology of mammalian reproduction appear to have been overlooked.

It is true that semen is deposited in the vagina which is lined by stratified, squamous epithelium, but to reach the ovum sperms have to ascend through the endocervix which is lined by tall columnar (picket) epithelium. This is less likely to be traumatized than the rectal mucosa of a catamite, but its penetration by sperms has been invoked in explaining the origin of carcinoma, while anti-sperm antibodies are common in the human female.

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Fetal viability

SIR — Dr Jansen's choice of definition of "previable" is not as plain as he thinks (*Nature* 26 April, p.768). The *de facto* legal definition of viability is currently 28 weeks in the United Kingdom. The National Health and Medical Research Council (NH & MRC) of Australia suggests 20 weeks; others suggest 22 or 24 weeks as the medical definition of viability. If there is "a point at which extrauterine existence is possible", it would appear to extend over an eight-week period, which is a mathematical contradiction. But, of course, there is no "point", not even 20 weeks, because NH & MRC had to provide a rider to this choice when it stated that "dissection of the fetus should not be carried out while a heartbeat is still apparent or there are other obvious signs of life".

Dr Goodhart's choice of definition (*Nature* 26 April, p.768) appears more reasonable but on analysis is seen to contain a major flaw. The span of human existence from conception until death may be divided into 9 months in a fluid environment and the remainder in an air environment. It is axiomatic to state that the fetus flourishes in its fluid environment, even if it may die in that environment. It is illogical, therefore, to relate the viability of the fetus to its unnatural extrauterine state, just as it would be illogical to relate the viability of those born to their ability to survive in a fluid environment once again.

The best definition of the term "viable" in relation to the embryo or fetus that I could find is "capable of maintaining life". The term "previable" adds nothing to our understanding of intrauterine human existence.

PATRICK W. GILL

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Biological control

SIR — May I add to the list of methods suggested for control of the fly in a domestic environment? Curiously, neither F.E.G. Cox[1] nor K.L. Bell[2] have considered the possibility of biological control There are several advantages to be gained from establishing a flourishing domestic population of the fly's natural predator, the spider. In particular, application of this means of control would overcome the moral dilemma posed by the choice between swatting the fly and forcibly expelling the insect into an often hostile world.

As in all habitats, it is important to maintain a correct ecological balance within the home or laboratory. Nevertheless, my proposal is preferable to a more drastic form of biological control recorded many years ago. As pointed out by R. Bonne[3], direct predation of flies by humans (particularly elderly females) would seem to be unwise. It can be the beginning of a surprising food chain which, if this anecdotal account is to be believed, can have serious medical consequences and can even result in death in extreme cases.

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2. Bell, K.L. *Nature* **308**, 684 (1984).
3. Bonne, R. *There was an Old Lady who Swallowed a Fly* (Southern Music Publishing Co. Ltd.).

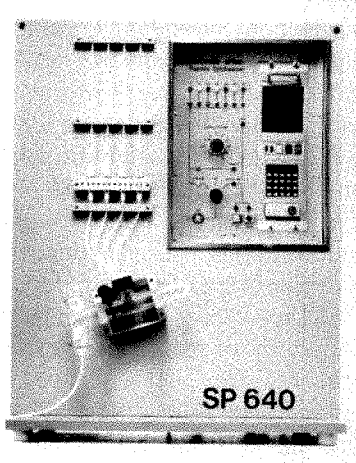
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SCANNING ELECTRON MICROSCOPY at MICRO 84

MICRO 84 takes place at the Bloomsbury Crest Hotel in London from July 9th to 13th 1984. MICRO is the largest conference and exhibition concerned with all aspects of light and electron microscopy; and any one microscopical technique, such as SEM, can be easily seen in the context of all the other, allied techniques, available. Today, the SEM is very much a microscopist's workhorse and there is a strong SEM theme running through the whole of MICRO. Some symposia are directed specifically to SEM, others have particular lectures which are relevant to SEM, while in still others complementary techniques are discussed. The following list highlights some of these lectures.

SEM OPIMISATION (Thursday/Friday)	The main objective of this symposium is "Getting the best out of your SEM". A series of tutorial lectures from acknowledged experts will cover the instrument, its operation, specimen preparation, image recording and image interpretation.
TUTORIALS IN MODERN ASPECTS OF MICROSCOPY (Wednesday)	This Symposium is concerned with microscopy and analysis of solid specimens. The lectures will not only cover SEM methods for the non-expert, but also Quantitative Microscopy, Scanning Optical Microscopy, Acoustic Microscopy, and Atom Probe Analysis.
STATE OF THE ART IN TEM AND STEM (Tuesday)	A series of tutorial lectures will deal with imaging and analysis of thin specimens. Among other things, the symposium is intended to update the knowledge of those not working directly with TEM or STEM.
ANALYSIS IN THE SEM (Friday)	In the sessions on X-ray microanalysis and cryo-techniques a number of the presentations specifically deal with preparation of specimens, including by low temperature methods, and their analysis in SEMs.
IMAGE RECORDING (Wednesday)	The symposium will include lectures on optimisation of scanning images, electronic processing of images, and photographic recording of images.
APPLICATION OF SEM (Throughout MICRO)	Throughout MICRO, many of the sessions will include lectures on applications of the SEM to structural observations and microanalysis. Topics including an SEM component include: interfaces in semi-conductors; automated SEM in environmental studies; automatic SEM particle analysis in forensic science; SEM in the study of ancient materials.
EXHIBITION (Throughout MICRO)	It is expected that more than 50 companies will be exhibiting their latest microscopes and ancillary equipment, covering the complete spectrum of instruments and techniques including, of course, full representation of SEM equipment.

Details of the MICRO 84 programme, and further information, are available from:-

The Administrator, Royal Microscopical Society, 37/38 St Clements, OXFORD OX4 1AJ. Telephone: (0865) 248768/721081

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Molecular biologists now using computer tools to locate functional patterns

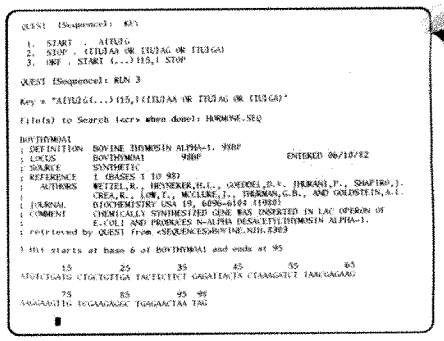
"As we learn more about the regulatory nature of the various genetic functional domains, and more about what...is recognized within those domains by cellular hardware, our comparative sequence and pattern identification algorithms will have to be generalized..."

—Sadler, Waterman, and Smith, NAR, April 11, 1983

Biological recognition searches

Regulatory sequences in DNA are inherently both redundant and complex. Consequently, a software tool that locates functional patterns must possess great flexibility. IntelliGenetics, the Palo Alto-based scientific software firm has developed this tool.

QUEST searches for biologically important consensus sequences, with any degree of specificity or redundancy. QUEST recognizes redundant patterns, and patterns of patterns. For example, comments in the NIH sequence library can be searched for the pattern "hormone" to generate a file containing hormone encoding sequences. This file can then be searched for three elements defining a gene: start, open reading frame, stop.



Combinations of keys enable QUEST to isolate complex patterns.

CLONER will also:

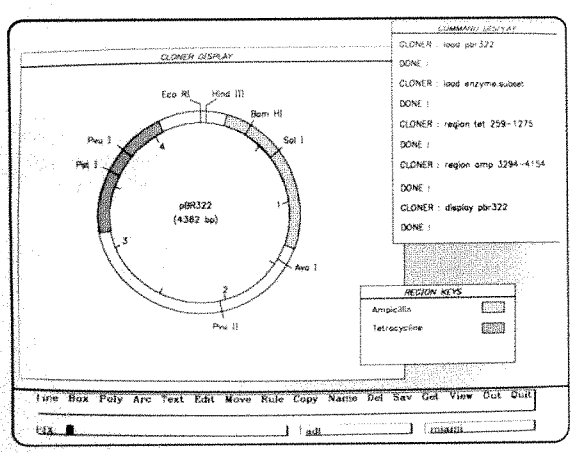
- store and retrieve maps of vectors and genes whether or not their nucleotide sequences are known
- carry genetic and functional information about regions of the maps
- manipulate map segments identified by restriction site names, by coordinates or by region information
- generate new maps when a vector is modified including all of the region information, restriction sites, gene locations and polarities
- determine whether regions with genetic function have been modified by electronic recombinations
- prepare copy-ready formal maps for reports, proposals or publication

The role of the desktop minicomputer in genetic engineering

Computers allow molecular biologists to gain new insights from their data. IntelliGenetics users are more productive scientifically because their computer tools eliminate many time-consuming tasks such as those associated with shotgun sequencing projects and restriction enzyme fragment mapping.

Microcomputers are useful for data editing and simple, unambiguous sequence searches. However, complex pattern searches require the power of a 32-bit computer.

All of IntelliGenetics' genetic engineering tools, plus databases are contained in a 32-bit UNIX computer: the BION Workstation. BION has been developed by IntelliGenetics to bring computer aided design to molecular biologists.



The bit-map display provides high-resolution maps and multiple windows.

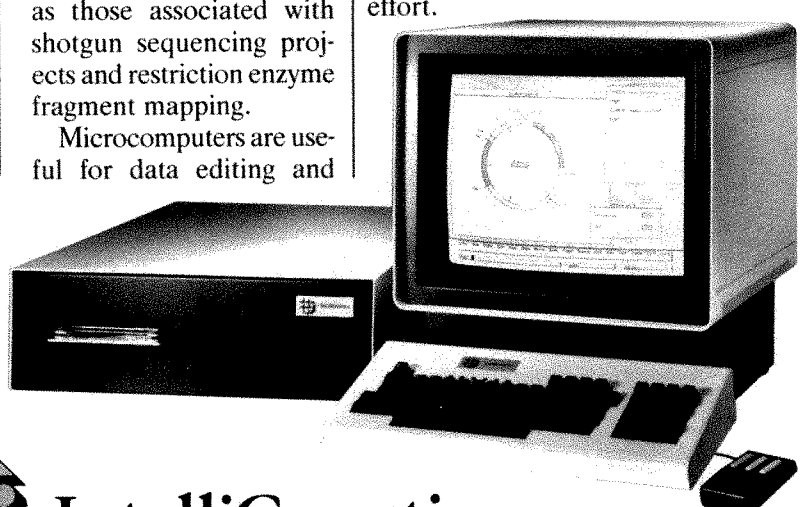
Artificial intelligence based graphics program

A new program currently being developed is a computer aided design tool called CLONER. With this program, the molecular biologist will plan and create new molecules by recombining DNA segments such as vectors, genes, promoters, and enhancer-segments.

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Blood group and socio-economic class

A report last year, based on a study of a British population, that A blood groups are significantly more common among members of the higher socio-economic groups (classes I and II) has generated a wealth of correspondence, some of which appears in what follows together with a reply from the original authors. The general conclusion seems to be that there can be no general conclusion.

BEARDMORE and Karimi-Booshehri[1] recently reported an association between ABO blood group phenotype and social class. They found that in both sexes the A phenotype was significantly more frequent and the O phenotype significantly less frequent in social classes I and II, the converse being true for social classes III, IV and V. In an attempt to replicate Beardmore and Karimi-Booshehri's findings we have analysed data obtained by the National Child Development Study (NCDS)[2].

The NCDS study commenced as the Perinatal Mortality Survey and involved all children born in Britain during the week 3–9 March 1958. At that time detailed obstetric data were obtained on each child[3,4]. In addition the mothers blood type (ABO and Rh status), her occupational status at commencement of pregnancy, and the occupational status of her father and of her husband were noted. The standard geographical region in which she lived and in which she was born were also recorded.

The occupational status of a married woman can be classified in several ways: (1) by her own socio-economic group (although this necessarily excludes a large number of women who are housewives); (2) on the basis of her father's occupation (when she left school); (3) on the basis of her husband's occupation (in 1958). We have used all three classifications in analysing the relationship between ABO blood group phenotype and social class.

Table 1 presents the breakdown of ABO blood group phenotype by social class and by migrational status. Natives were defined as those born and still living in the same region; all other individuals were classified as migrants. The regions were defined as the 11 standard regions of Britain[5].

For all three classifications of social class we found no evidence to support the notion of either heterogeneity between migrants and natives ABO phenotypic distributions (see Table 1) or an excess of the A phenotype in social classes I and II.

There are several possible reasons for our failure to replicate Beardmore and Karimi-Booshehri's results:

Table 1 Percentages of ABO blood group phenotypes by social class (three classifications) in natives and migrants in Britain

Classification		Blood group	Social class I	II	III	IV	V	Total No.	%
Mother's own	Migrant	A	41.7	39.4	44.4	50.0		327	41.6
		O	50.0	49.8	43.7	42.9		379	48.2
		B	7.6	6.9	9.3	5.4		58	7.4
		AB	0.7	3.9	2.6	1.8		23	2.9
		Total no.	144	436	151	56		787	
	Native	A	40.8	42.3	41.5	47.6		1,329	42.2
		O	47.8	46.6	48.6	44.7		1,479	47.1
		B	8.0	8.5	8.2	5.8		257	8.2
		AB	3.3	2.7	1.7	1.9		77	2.5
		Total no.	299	1,832	805	206		3,142	

χ^2 testing:
ABO by social class (migrant) $\chi^2(9) = 8.359$ $P = 0.498$
ABO by social class (native) $\chi^2(9) = 7.226$ $P = 0.614$
ABO by migrational status $\chi^2(3) = 1.306$ $P = 0.728$
Migrational status by social class $\chi^2(3) = 54.963$ $P < 0.001$

			I	II	III	IV	V	Total No.	%
Father's social class	Migrant	A	36.2	38.8	41.5	41.7	45.7	718	41.1
		O	59.4	53.0	46.0	47.4	42.6	835	47.9
		B	0.0	4.7	8.7	7.8	7.8	129	7.4
		AB	4.3	3.5	3.7	3.2	3.9	63	3.6
		Total no.	69	317	882	348	129	1,745	
	Native	A	40.4	40.9	41.2	43.6	41.7	2,538	41.8
		O	47.5	47.4	47.5	45.4	45.0	2,836	46.7
		B	10.1	8.5	8.4	8.2	10.3	521	8.6
		AB	2.0	3.2	2.9	2.8	3.0	176	2.9
		Total no.	99	709	3,054	1,585	624	6,071	

χ^2 testing:
ABO by social class (migrant) $\chi^2(12) = 17.320$ $P = 0.138$
ABOby social class (native) $\chi^2(12) = 6.551$ $P = 0.886$
ABO by migrational status $\chi^2(12) = 5.062$ $P = 0.167$
Migrational status by social class $\chi^2(4) = 110.064$ $P < 0.001$

			I	II	III	IV	V	Total No.	%
Husband's social class	Migrant	A	37.8	42.2	40.9	45.4	41.2	805	41.4
		O	51.9	46.8	46.4	45.4	43.8	910	46.9
		B	8.9	8.7	9.3	6.9	12.5	173	8.9
		AB	1.4	2.3	3.4	2.3	2.5	54	2.8
		Total no.	214	393	993	262	80	1,942	
	Native	A	43.4	43.4	41.4	40.8	39.2	2,855	41.5
		O	48.4	45.4	46.8	48.6	48.6	3,243	47.1
		B	5.5	7.2	8.9	8.1	8.7	576	8.4
		AB	2.7	4.0	3.0	2.5	3.5	209	3.0
		Total no.	256	917	3,991	1,293	426	6,883	

χ^2 testing:
ABO by social class (migrant) $\chi^2(12) = 9.350$ $P = 0.673$
ABO by social class (native) $\chi^2(12) = 13.629$ $P = 0.325$
ABO by migrational status $\chi^2(3) = 0.874$ $P = 0.832$
Migrational status by social class $\chi^2(4) = 248.342$ $P < 0.001$

Values in parentheses are d.f.

(1) We have looked only at women, whereas Beardmore and Karimi-Booshehri considered both sexes. Nevertheless it is clear from their analysis that their effect is found in both sexes to the same degree. We have attempted to repeat their study by considering both natives and migrants. If this restriction is removed, the sample available for analysis increases, but there still remains no association between ABO blood group status and social class.

(2) The NCDS is a sample of the breeding population of Britain whereas the blood donor data will also include non-breeders. However, it is more likely that the sampling procedure used by the NCDS generates a truly random sample, whereas it is not inconceivable that blood donor data could be biased.

(3) Some evidence of nonrandomness in the blood donor data can be obtained from analysis of the social class distributions. Comparison of each data set with the Registrar General's social class distributions indicates that the NCDS data do not differ markedly from expectation whereas the donor data reveal a significant excess of people in social classes II and V (about 5% and 12% respectively) and relative deficiency of 21% in social class III. There is also marked heterogeneity between NCDS and blood donor samples, for example, using the husband's occupation $\chi^2 = 1{,}746.855$, $P < 0.0001$ (d.f. = 6). Restricting the comparisons to the Registrar-General's standard regions of East and West Riding and south-west England does not affect the above conclusions.

We also note that Beardmore and Karimi-Booshehri found an association only between ABO phenotype and social class and not between migrational status and ABO phenotype, despite a clear association between migrational status and social class.

(4) The ABO phenotype frequencies differ between samples. This is to be expected since we have analysed data from Britain as a whole rather than two geographical regions. However, if we restrict our analysis to the two Registrar-General standard regions mentioned above there is no heterogeneity in ABO phenotypes between samples. Even with this reduced sample we can find no evidence to support Beardmore and Karimi-Booshehri's results.

We have also examined the relationship between social mobility and ABO phenotype, classifying mothers on the basis of her own and her father's social class, as either upwardly mobile, downwardly mobile or non-mobile. Again there was no evidence that this intergenerational social mobility was related to ABO phenotype ($\chi^2 = 1.457$, $P = 0.962$, d.f. = 6).

We thank Mr K. Fogelman, and the National Children's Bureau, for allowing us to re-analyse the data obtained by the National Child Development Study, and Mr E. Roughley, and the Social Science Research Council's Survey Archive for supplying the data.

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IN a recent article[1], Beardmore and Karimi-Booshehri showed that among 10,000 blood donors from south-west England and Yorkshire there were marked differences in the ABO blood group distribution with social class. The study by Mascie-Taylor and McManus shows an analysis of women who gave birth in the 1 week's national birth survey in 1958. Here we analyse data from a subsequent birth survey. The data relate to 14,384 women resident in Great Britain and who gave birth in the week 5–11 April 1970. The data were collected in the British Births survey[2,3] sponsored by the National Birthday Trust Fund. Well over 95% of all births in England, Scotland, Wales and Northern Ireland were included.

The ABO blood groups of these mothers were compared with their social class, based on the occupation of the husbands, using the Registrar General's Classification of Occupations[4]. Unmarried mothers and those whose husbands' occupations were unclear were omitted ($n = 415$). Ths distribution of ABO blood groups using this classification is shown in Table 2. As shown for the 1958 survey, there was no significant difference between the social classes.

This classification uses the husband's occupation. We did not use that of the woman herself since married pregnant women are often housewives or are only in part-time employment. Information was available, however, concerning the

Table 2 Distribution of ABO blood groups of mothers delivering during 5–11 April 1970 by social class (based on husband's occupation)

Blood group of mother	Social class I	II	III	IV	V	All known
O	368 (48.0%)	822 (47.0%)	4,110 (47.6%)	1,099 (49.2%)	484 (49.0%)	6,883 (47.8%)
A	300 (39.1%)	727 (41.4%)	3,482 (40.3%)	833 37.3%)	373 (37.7%)	5,715 (39.7%)
B	73 (9.5%)	160 (9.1%)	792 (9.2%)	231 (10.3%)	108 (10.9%)	1,364 (9.5%)
AB	26 (3.4%)	45 (2.5%)	258 (2.9%)	70 (3.2%)	23 (2.4%)	422 (3.0%)
All known	767 (100.0%)	1,754 (100.0%)	8,642 (100.0%)	2,233 (100.0%)	988 (100.0%)	14,384 (100.0%)

$\chi^2 = 16.1$, d.f = 12, not significant.

Table 3 ABO blood groups according to highest educational qualification of mothers

Highest educational qualification	Blood group O	A	B	AB	All known
None	2,884 (46.9%)	2,510 (40.8%)	574 (9.4%)	180 2.9%)	6,148 (100.0%)
Vocational	778 (49.0%)	623 (39.2%)	142 (9.0%)	45 (2.8%)	1,588 (100.0%)
O-level or equivalent	932 (47.3%)	816 (41.5%)	162 (8.2%)	59 (3.0%)	1,969 (100.0%)
A-level or equivalent	186 (47.2%)	154 (39.1%)	47 (11.9%)	7 (1.8%)	394 (100.0%)
SRN or Certificate of Education	272 (49.6%)	211 (38.4%)	52 (9.4%)	14 (2.6%)	549 (100.0%)
University or college degree or other higher	181 (44.8%)	173 (42.8%)	40 (9.9%)	10 (2.5%)	404 (100.0%)
All known	5,233 (47.3%)	4,487 (40.6%)	1,017 (9.2%)	315 (2.9%)	11,052 (100.0%)

$\chi^2 = 12.7$, d.f = 15, not significant.

educational qualifications of the mothers of the surviving children who were followed up at the age of 5 yr.

As shown in Table 3, there is no evidence for a trend. The evidence of Beardmore and Karimi-Booshehri[1] would suggest that, as the educational qualifications increased, so the proportion of blood group O would decrease, and that of A would increase. True, in our data there were slightly more women with university degrees who had blood group A but this was far from statistically significant ($\chi^2 = 0.5$, d.f = 1).

What, then, might account for the differences between the two studies? Both, of course, have selection biases—in Beardmore and Karimi-Booshehri's data, blood donors only are included and housewives are excluded. In our study, women who are infertile will be excluded and those with high fertility will be more likely to be represented. Although we have no information on the ABO distribution of the former, it has been shown[5] that there is little variation in the distribution of ABO blood groups with the number of pregnancies the woman has already had.

From the data presented here we suggest that, although perhaps true of non-migrant blood donors in Yorkshire and the south-west of England, the finding of more blood group A amongst the upper social classes cannot be extrapolated to the female population as a whole. We know that data from the 1958 birth survey (see above) substantiates our findings. We therefore suggest that the original results[1] are likely to have been due to one of those random statistical variations which must occur by chance.

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FROM a χ^2 analysis done on an historic (1953–63) data base, Beardmore and Karimi-Booshehri[1] conclude that attributes influencing occupational type, social mobility and social class result from pleiotropic effects of the ABO alleles (or genes closely linked with them). In the most cogent of their theoretical citations, Herrnstein[2] suggests a possible heritable relationship between social class and intelligence. The authors also cite Arthur Jensen's work[3]. Many recall the social upheaval which accompanied those writings a decade ago and seek to be very parsimonious when making ties between genetics and social class. In that context, I suggest that while the author's hypothesis may be true, it is not yet clearly indicated. Their extensive data seem better interpreted with the notion that there is no direct path from these genes to the behavioural phenotypes. In earlier work concerned with single gene substitutions and behaviour, I have supported the position that while single genes may sometimes have major effects, they often affect many forms of behaviour and that particular psychological traits are frequently influenced by many genes[4]. In the service of that theoretical viewpoint, I have reassembled and recalculated the data on ABO phenotypes as they assort by socio-economic group.

The highly significant outcomes reported[1] do represent real findings with regard to differential distribution of the ABO blood phenotypes across the five socio-economic job categories chosen. However, the authors have repeatedly combined categories to give classes I+II and III+IV+V. They also present the results by comparing the frequency of A phenotypes to the O+B+AB phenotype combination across the socio-economic categories. According to conventional usage in the behavioural sciences[5] information is lost by these procedures. Groups may be (sensibly) combined for an analysis, of course, but this is normally done in order to meet the restrictions regarding minimum expected frequency requirements[5]. With these data, even the least frequent phenotype (AB) provides sample sizes that are adequate for independent χ^2 tests involving all five job categories. Then, since the χ^2 distribution is insensitive to the effects of order, a presentation of the contingencies by cells shows up areas of particular effect. Table 4 gives the obtained frequency divided by the expected frequency ratios for the four ABO phenotypes. It will be seen that for each phenotype there are one or two contingencies which indicate either marked under- or over-representation. The A phenotype is heavily over-represented in job category I and it is marginally high in category II. Phenotypes O, B and AB are all much under-represented in category I but show very different distribution patterns across categories II to V. The AB phenotype is low for category II as well as I and is notably high for category V. The test for the contingencies of Table 4 is significant: χ^2 (d.f. = 12) = 83.03, $P < 0.001$.

This significant statistical outcome, and those of the original report, may represent what can be called 'behavioural pleiotropy'. They do not confirm true pleiotropy, as that term has had meaning in formal genetics. In his classic text Grüneberg[6] discussed genuine and what he called 'spurious' pleiotropy. The rule is that in genuine pleiotropy a single gene controls two or more different traits which it produces directly by the use of different mechanisms. In spurious pleiotropy the gene does two or more things through the same mechanism or else has one effect which causes other things to happen. For example, in mice, A^y, a single gene which has genuine pleiotropic structural effects, for example, increased fat deposition, skeletal enlargement, linear size increase, also shows behavioural (spurious) pleiotropy in that it is also associated with the amount of explorary activity exhibited by C57BL/6J mice[7]. The genuinely pleiotropic traits sum to effect greater body weight which, in turn, accounts for 48% of the variance in activity when $A^y a$ mice are compared with *aa* controls[7]. Thus, the behavioural pleiotropy can be clearly shown statistically but has no genetic basis of its own.

Table 4 Obtained frequency/expected frequency ratios in the total data set of Beardmore and Karimi-Booshehri

Blood phenotype	Socio-economic category I	II	III	IV	V
A	1.34	1.09	0.96	0.99	0.92
O	0.74	0.91	1.06	0.98	1.06
B	0.74	1.08	0.92	1.10	1.02
AB	0.78	0.84	1.00	0.99	1.18

So finally, if we evaluate all of the ABO phenotypes concurrently, both the statistical and the definitional points work together in our understanding (and lack of understanding) concerning how the class differences reported[1] come to be associated with the ABO blood antigen system. We see that one blood phenotype is at comparative advantage for employment, another (the AB phenotype) is at comparative disadvantage, and that (especially in categories II–V) there is a great deal of intra-phenotypic variability across the socio-economic groups. This last point tempers the positive finding that the greatest part of the overall effect is seen at the extremes of the socio-economic continuum.

Thus, it seems a long way from the statistical outcomes to the privilege (or poverty) of class-based experiences, activities, comfort and substance as these subjects have matured from birth to their time of blood donation. In short, when we can show that some genotypically influenced mechanism intervenes between the socio-economic continuum and the discrete phenotypes of the ABO blood system, then we can show pleiotropy. When no mechanism is evident and a statistical outcome is our only basis, then social, cultural or other environmental influences seem more likely to contribute much of the variance to the behavioural pleiotropy found.

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THE conclusion of a recent paper[1], that a pleiotropic effect of ABO alleles or linked genes can influence occupational type, seems to be premature. The authors assume that occupational classes are homogenous from an ethno-historical point of view, assortative mating among social classes is not significant, the ABO distribution of donors is similar to that found in the general population, selection on the ABO system is not differently operating in social classes and finally, that the occupation as a variable is the most representative one that defines the stratification of a society.

In Chile, as the authors mention, there is a clear socio-ethno-genetic cline which originated at the Spanish Conquest in 1541 [2,5]. The highest socioeconomic strata of urban areas have gene frequencies for ABO, Rh and other genetic markers similar to those found in Europe, the lowest ones have gene frequencies similar to those expected for a half European–Chilean Aboriginal admixture; middle classes have intermediate values[6]. This cline is present independently of the last two or three generations of European inmigrations[7,8]. Thus the problem is to explain why such a cline has persisted for 17 generations. Aboriginal people disappeared a few generations after the Spanish Conquest. Even 20% socioeconomic random mating should have cut down these gene differences but a strong socioeconomic assortative mating was found in the population of Santiago[9]. This seems to be the explanation for the stability of the socio-genetic cline. There is no reason to assume that a similar mechanism is not operating in England. Since geographical population heterogeneity (like that found also in Chile) due to invasion, settlement and migration is accepted by the authors, it would be necessary to demonstrate that ethnic heterogeneity and assortative mating among occupational classes cannot explain the observed ABO heterogeneity, before accepting the authors' hypothesis.

Blood donors may not represent either the social or the genetic structure of their populations of origin. This could not only be due to higher requirements for O and Rh^- blood groups as the authors assume. If the relationship between ABO and diseases is accepted, and donors are relatives or friends of patients that need transfusions, the ABO distribution of donors may differ from that of the whole population. The authors do not describe the system to call for blood donors. Relatives of patients would increase distortions due to the ABO–disease relationships, colleagues or co-workers of patients would yield a distribution similar to that of the patient social class. These two types of calling donors or other ones, may be differently distributed in occupational classes, thus leading to different ABO distributions among them. We could not use the donors ABO distribution in the high socioeconomic classes because it was different from the mother–infant distribution in a study on socio-economic assortative mating[9]. Moreover, ABO-associated diseases might interact with social classes in an unpredictable way. Furthermore, natural selection operating on the ABO system[10] may differ among social classes, not necessarily at present, but along the history of these classes.

Table 1 of ref. 1 shows that in class I, A migrant donors are less frequent than A native donors (z for proportions[8] = 2.14, $P<0.017$ in south-west England; $z=1.2$, $P<0.11$ in Yorkshire; $z=2.25$, $P<0.013$ in both places together); thus non-A migrant donors are fitter than non-A native donors to be found in class I. This suggests rather a socio-genetic interaction and not a simple gene action on work abilities.

Even accepting that a genetic action associated with ABO system influences the probability of reaching a determined social class, it is not possible to conclude that this gene action is related to skilled/creative or skilled/repetitive occupations. Several other social variables may influence the occupational class an individuals belongs to, such as culture, power, prestige, income and life-style, the latter being rather properties of class and conditioning individual occupation or social mobility. If so, the ABO–occupational class relationship may be true, but only as a circumstantial association.

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BEARDMORE and Karimi-Booshehri[1] conclude that "The only apparently reasonable explanation" for the distribution of the I^A allele is that it "confers on those who possess it... abilities significant in determining socio-economic class" (pleiotrophy and linkage being gratuitous qualifiers). No less disingenuously, they assert that "social mobility in the United Kingdom has been, for some generations, a factor which would tend to homogenize initially different gene pools". The question is for how many more generations did social immobility increase the heterogeneity of gene pools? An order of magnitude more than "some"? And how quickly would Karimi-Booshehri and Beardmore expect that heterogeneity to disappear? Figuratively overnight? Apparently.

People who inherit wealth do not start at the starting-line with the rest of the runners, nor do their children or their children's children. It is reasonable to speculate that the present distribution of the I^A allele is one time-frame taken from a gradual sequence of allelic desegregation—the remnant of centuries of tradition during which the mere possession of wealth and social class was enough to ensure the maintenance of same—even in the face of a conspicuous lack of ability, genetic or otherwise.

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BEARDMORE AND KARIMI-BOOSHEHRI REPLY—Mascie-Taylor and McManus's interesting findings on a sample of mothers fail to confirm ours. They consider a number of possible reasons for this. However, they do not take age into account. As the majority of children are born to mothers in their twenties or early thirties there is massive overrepresentation of younger cohorts in their sample. Thus, quite apart from the omission of non-breeders, their sample could not be said to be representative of the female segment of the population in a social sense.

A second important difference in sample compositions between the two

studies is that in Mascie-Taylor and McManus' data for geographical regions are pooled. As there are both average genetic and average socio-economic differences between regions the pooling makes comparisons very difficult.

It is true that our data are nonrandom in terms of social class proportions. We are unable to judge the effect of our exclusion of cases with no or ambiguous occupational labels but it would not be surprising if this produced under-representation of class III especially as one of the commoner labels excluded was 'civil servant'. However there is no obvious mechanism by which such nonrandomness should lead to the results observed.

Mascie-Taylor and McManus are concerned that we have not demonstrated an association between migrational status and ABO type and believe that this may imply mis-classification of social class. Our view is that we have, in this study, been at very considerable pains indeed to avoid mis-classification and that mis-classification is likely to have been at a very low level and lower than other relevant studies. Furthermore it does not follow that an association between migration and ABO type would be detectable as the influences on migration and social class are by no means coincident.

There is a major difficulty in comparing the data of Golding *et al.* with ours. This is that they use the social class of husbands to assign social class to the women in their surveys. There is a correlation between social class of spouses but it is by no means perfect. We were meticulous in our assignment of social class from occupational type. It is interesting to note that recalculation of the total 1958 data ($n = 13{,}969$) collected in the First Perinatal Mortality Survey (of which the data given in Table 1 are a subset) shows the ratio A/O+B+AB to be almost identical in form with our Fig. 1 (Spearman rank correlation in both cases is 0.9, $P \simeq 0.05$). The pattern for the 1970 data quoted by Golding *et al.* is different and indeed the χ^2 testing homogeneity of the 1958 and 1970 data on ABO phenotype frequencies is very close to the 0.05 level of significance. The presence of Northern Ireland data in only the 1970 sample makes it difficult to assess whether it is this or temporal changes which may be responsible for the difference. The 1958 and 1970 samples also differ from each other in social class composition and this, too, complicates comparisons.

Hawkins raises two issues. One concerns the difference which he sees between 'true' and 'spurious' pleiotropy. This is a semantic point. While there may be a case for making distinctions as to types of pleiotropy, many geneticists, including the writers, would subscribe to the view that all effects (whether these affect anatomical, behavioural, biochemical, reproductive or viability aspects of the phenotype) of gene differences can legitimately be considered pleiotropic effects. The second point is concerned with the method of statistical analysis. Pooling of data always leads to loss of information but the main conclusions are unaffected in this example whether we pool or not.

We have partitioned the table into a series of 2×2 tables from each corner. Irrespective of the starting point, it is clear that O, B and AB do not differ over socio-economic class and therefore the table is collapsible in the sense of Bishop, Fienberg and Holland[1]. Thus, only the differences A/(O+B+AB) over social class are relevant. Using the partition method of Daly[2], comparisons which show significance (after pooling blood groups) are those between classes I and II and (I+II) against the rest. Clearly, the significance of the first requires further analysis, but the second accounts for about 50% of the overall χ^2 and cannot be controverted. We also noted a significant effect for classes (I+II+III+IV) against class V, but this only confirms the original conclusions.

Valenzuela takes the view that assortative mating within social classes derived from ethnically different populations is the proper explanation of our results. While ethnic heterogeneity is demonstrable in the Chilean population it is not apparent in the population from which our data are drawn. The difference in the proportions of the A phenotype in migrants and natives is, we think, significant and needs further investigation. In no way do we subscribe to the view, which Valenzuela appears to think we hold, that non-genetic factors are unimportant in determining occupation or other indices of social class.

The other question Valenzuela raises is that of how random a sample of a population is obtained by collection of blood donors. There is a clear need to test this and we hope shortly to examine this question using large samples of newborn and donors from the same region. Nonrandomness, if it occurs, will most likely take the form of over-representation and under-representation of particular phenotypes and the influence of patients on recruitment to donor panels in the UK is unlikely to be significant. One relevant set of data is available in which the distribution of ABO phenotypes can be compared in donors and schoolchildren in one locality. This comes from the work of Allan[3] and is given in Table 5. The phenotype distributions in the two samples agree very well and the very small difference between the two is in the expected direction of slightly more O in the donor sample. It is, of course, possible that such samples differ in social composition.

Table 5 Proportions of ABO phenotypes in two samples from Aberdeen

Sample	Phenotype O	A	B	AB	*N*
Donors	50.27	35.46	10.87	3.40	7,092
Schoolchildren	49.75	36.16	10.72	3.37	17,060

Hartung asks an important question which cannot be answered with assurance. We have, however, with the collaboration of Dr R. Gilbert, attempted to make some crude estimates which are necessarily based on a number of debatable assumptions. Let us suppose that mixing of two populations each polymorphic for genes B^1 and B^2 with the frequencies of B^1 being 0.35 and 0.15 respectively occurs. Even if the gene flow between the two populations ('classes') is as low as 2%, the two classes will have frequencies of B^1 of 0.26 and 0.24 respectively in 56 generations. With 5% gene flow the same frequencies are achieved in 22 generations. Using 25 yr as the length of a generation, the time lapse and gene frequencies are perhaps reasonably applicable to populations at the time of the Saxon invasions of the British Isles which had considerable consequences for power and class. The important point is that these levels of gene flow are very low and it is not unreasonable to suppose that effectively much higher levels of gene flow have operated both in the recent and more distant past, although there is no obvious way of testing this view.

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Psychoimmunology before its time

That there is probably a link between the central nervous system and the immune system is easily accepted. The doubt is whether enough is yet known to sustain people's hopes of explanation.

THE notion that emotional disturbance may trigger off physical illness is in no sense new. That people may be driven by adversity into decline is a familiar theme in literature. Strictly speaking, the phenomenon is distinct from that of psychosomatic illness such as Freud's well-documented cases of hysterical paralysis in which, in Freudian terms, the illness is a kind of talisman for the emotional disturbance. Instead, the physical consequences of emotional shock may become manifest in normal people even if they are more spectacular among those with an underlying emotional disturbance.

Over two decades, Professor G.W. Brown of Bedford College, University of London, has been collecting evidence that what he calls life-events (imprisonment, relocation or personal bankruptcy, for example) may provoke illness in outwardly normal people. The speed with which a person's death will follow that of his or her spouse is the most familiar occurrence of this kind. The pattern is familiar, but the mechanism is unknown.

That, at least, has been the state of affairs until quite recently. Now, as some of the new generation of medical students know perhaps too well, there is emerging a speciality called psychoimmunology — a means of explaining such phenomena as the increased risk of death on bereavement in terms of the disturbance caused to the immune system in the wake of emotional shock or deprivation. The difficulty, for the rest of us, is that of knowing just what weight to give to these accounts.

That there should be a functional connection between the central nervous system and the immune system is not in itself surprising. In animals, neurophysiologists now have a lot to say about the way in which the brain controls the system of locomotion, while the regulation of the reproductive system in mammals by means of the peptide hormones involved in the regulation of the pituitary gland by the hypothalamus is being understood in ever finer detail.

A kind of connection between the central nervous system and the immune system is made plausible by several unconnected observations, not the least of which is that some types of the lymphocytes involved in the body's immune response to, say, bacterial infections are found to carry receptors which recognize simple peptide hormones also found in the brain.

Some of the connections have been reviewed by David Maclean and Seymour Reichlin (*Psychoneuroimmunology* **12**, 475; 1981), and can plainly take several different forms. Maclean and Reichlin offer the interaction of the hypothalamus and the pituitary as the most interesting source of materials that may modify immune function. Some possible interactions are direct, as with the pituitary hormone that appears directly to stimulate the development of cells in the thymus, the organ in which T-cells become recognizably different from cells that produce antibodies. But the best-known routes by which pituitary hormones may affect the immune system are indirect, involving the stimulation of the adrenal cortex by hormones such as adrenocorticotropic hormone.

This imbalance is unlikely to persist for long. As techniques improve for distinguishing between T-cells in different stages of their maturation and for sustaining them in culture, there has naturally been an upsurge of interest in the measurement of response by lymphocytes to hormones of various kinds. It seems to be established that the steroid hormones of the adrenal cortex more markedly stimulate lymphocytes at early rather than late stages in their differentiation, which may suggest how the composition of the circulating lymphocytes may be controlled by peptide hormones.

And then, it seems, some peptide hormones stimulate T-cells to produce lymphokines (such as interleukin-2 but also interferon) and others have the opposite effect. The more radical psychoimmunologists talk as if there is no state of mind which is not faithfully reflected by a state of the immune system.

Others go further. J. Edwin Blalock of the University of Texas at Galveston, writing in the *Journal of Immunology* in March (**132**, 1067; 1984) under the title "The immune system as a sensory organ", argues that the interaction between the central nervous system and the immune system must be reciprocal, with peptide hormones of the pituitary and lymphokines secreted by lymphocytes having similar functions in the blood and with antigenic stimuli such as different kinds of bacterial infections producing physiological and even behavioural changes in the infected animal. He does not quite say that the emotional consequences of measles and tuberculosis infections should be distinguishable — but of course, they are.

The other line of inquiry that has helped put psychoimmunology on its feet are experiments with whole animals. In many ways, these are adjuncts of the classical investigations of the effects of stress on the system of adrenal hormones and thus on blood pressure, heart rate and so on.

In one typical investigation (Laudenslager, M.L. *et al. Science* **221**, 568; 1983), groups of a dozen rats were given electric shocks of two kinds, and the sensitivity of their lymphocytes to artificially provoked cell division by the materials called mitogens assessed. The more seriously stressed rats were found to have the least easily stimulated lymphocytes at the end of the experiment.

Surprisingly, very similar investigations can be mounted with people. Thus S.J. Schleifer *et al.* have tackled directly the question of the immunological consequences of bereavement by means of a prospective study among men whose wives had been found to have breast cancer. Those who were eventually widowed have been reported (*J. Am. med. Ass.* **250**, 374; 1984) to have had circulating lymphocytes less responsive to the effect of mitogens, just like the rats given electric shocks.

Investigations such as these, while time-consuming and costly, at least have the merit of being practicable and, once mounted, easy to interpret. The snag is that statistically significant samples are accumulated only slowly, and at a high cost.

Why, in these circumstances, does there persist a stout band of near-sceptics convinced that too much is being made, at this stage, of the explanatory value of psychoimmunology? There are several reasons, not the least of which is that very similar lines of argument, more accurately, hypotheses, have been used within living memory speciously to suggest links between a person's chance of developing cancer and his or her state of mind.

Second, because knowledge of T-cells and their function is growing so quickly, it is necessarily difficult to design systems for assaying the effects of hormones, or of states of mind, on lymphocytes that are reproducible and significant.

Third, one rather crucial question seems to have been left for too long aside. If it should be that a person's state of mind, say grief, may affect the quality of his immune system, how does it then transpire that he or she will be more likely to die of heart attack or stroke or even in a road accident? In short, the explanation is likely to be part of a more complicated tale. **John Maddox**

Evolution

Palaeontology at the high table

from John Maynard Smith

THE Tanner lectures, recently delivered by Stephen J. Gould of Harvard University, and followed by a panel discussion*, provide an opportunity to assess the current contribution of palaeontology to evolutionary theory. It might be supposed that this contribution would be crucial, but, at least until recently, that has not been so. The palaeontologist G.G. Simpson was one of the main architects of the 'modern synthesis' that emerged in the 1940s, but his role was to show that the facts of palaeontology were consistent with the mechanisms of natural selection and geographical speciation proposed by the neontologists (a term used by palaeontologists to describe the rest of us), rather than to propose novel mechanisms of his own. Since that time, the attitude of population geneticists to any palaeontologist rash enough to offer a contribution to evolutionary theory has been to tell him to go away and find another fossil, and not to bother the grownups.

In the last ten years, however, this situation has been changed by the work of a group of palaeontologists, of whom Gould has been a leading figure. In his lectures, he stressed two theoretical modifications to the Darwinian scheme — 'punctuated equilibria' and a hierarchical view of evolution. Punctuational theory holds that most evolutionary change occurs when lineages split — that is, when a single species gives rise to two. Between such events, species remain morphologically unchanged, often for millions of years: they remain in 'stasis'. In itself, the theory says nothing about the mechanism of the changes when they do occur, other than that they are rapid in geological terms: they could still be slow to a geneticist, and brought about by the familiar process of natural selection. As J.S. Jones (University College, London) remarked in an earlier discussion, one man's punctuation is another man's gradualism.

Little was said in Cambridge about the empirical evidence for punctuation. Gould treated it as a fact the whole world knows: as something proved by Aunt Jobisca's theorem. As an outsider, I am persuaded that morphological evolution proceeds at very different rates at different times, but can see little evidence that rapid change is necessarily associated with the splitting of lineages (although one would expect this often to be the case, when part of a species enters a new ecological niche). What is new is the emphasis on stasis. As David Wake (University of California, Berkeley) pointed out during the discussion, the existence today of pairs of species which are morphologically indistinguishable, or almost so, but whose proteins have diverged sufficiently to suggest that they have been separate for millions of years, supports the reality of stasis. Stasis, then, is a phenomenon that calls for an explanation.

Geneticists and palaeontologists tend to differ in the explanations they prefer. Population geneticists argue that if a species does not change, this is because it is subject to 'normalizing selection' — that is, selection that eliminates the extreme phenotypes and favours the norm. They have two main reasons for this belief. First, if directional selection is applied to almost any characteristic, in nature or in the laboratory, the population will change. Second, most species with a wide geographical range do vary in structure and behaviour, sometimes to such an extent that populations from different regions would be regarded as different species if they were not connected by a series of intermediate forms. (In case anyone supposes that membership of a species is decided unambiguously by hybridization, it is the case that different species often produce fertile hybrids, and populations of the same 'species' often prove, on investigation, to produce sterile or inviable hybrids.)

In reply, Gould asks how it could possibly be the case that normalizing selection could favour the same phenotypes over long periods, during which the environment may change drastically. Instead, he proposes a concept of species stability arising from developmental constraints. The constancy of a species would thus resemble the constancy of a chemical element: it would represent a stable state of matter, which can be changed only by an unusual event. C.H. Waddington's idea of 'canalization' has been quoted in support of this view. Waddington did indeed point out that the typical 'wild-type' morphology of a species is usually well buffered against disturbance during development, whereas the phenotypes of individuals with a given mutation are highly variable between individuals. However, for Waddington, the uniformity of the wild type was itself the product of normalizing selection, and not a manifestation of an intrinsically stable state.

Hierarchies

The debate about stasis and punctuation will no doubt continue. Gould's second thesis concerned the hierarchical structure of evolution. Darwin, he argued, thought of natural selection as favouring some individual organisms at the expense of others. However, selection, and also chance, can be effective at two other levels, namely the gene and the species. At the level of the gene, we now know that there are processes (gene conversion, unequal crossing-over, transposition) that enable genes to multiply out of phase with the organisms in which they find themselves. It follows that a gene can increase in frequency by virtue of its ability to multiply horizontally within an individual, and not only by virtue of its effects on the individual's phenotype.

Two points were made about this level of selection during the discussion. John Fincham (Cambridge University) pointed out that since transposons are in effect parasites, we can expect them to evolve 'self-restraint', as some parasites do: it does not pay to kill the goose that will transmit you to future generations. A more fundamental disagreement was raised by Gabriel Dover's (Cambridge University) claims for 'molecular drive' — a portmanteau term for the three processes listed above. I may give an unfair account of this disagreement, because I was one of the protagonists. Dover asserted that there is nothing that natural selection can do that cannot be done by molecular drive. I argued in reply that this is to overlook the fact that natural selection can generate highly improbable adaptations at the organismic level, whereas molecular drive cannot. As I see it, Dover's view would throw out the Darwinian baby with the bathwater.

Species selection

Palaeontologists can contribute little to this debate, but have a lot to say about the level of species selection. The concepts can best be explained by some examples. First, during the Cretaceous, the Volute gastropods gradually replaced their competitors. A plausible explanation is that Volute development did not involve a stage of mobile planktonic larvae, whereas the development of their competitors did. As a result, geographical speciation was more frequent among Volutes, and the number of species increased. In effect, Volute species had a higher 'birth rate'. If this explanation is correct, it applies the concept of natural selection, but replaces individuals by species as the units selected.

Now consider a second example, the mammalian secondary palate. This is a structure that enables a mammal to breathe and chew at the same time. Gould would agree that such a structure (together with associated changes in teeth, jaw muscles and articulation) could arise only by natural selection at the level of individuals. Suppose, however, that mammalian species came into competition with reptiles lacking these adaptations. If the mammals outcompeted the reptiles because they could chew better, then mammalian species would replace reptilian ones. However, Gould would not call this species selection, because the success of the mammals would depend on something individual animals do (chew) and not on something species do: in contrast, in the Volute example, speciation is something species and not individuals do.

* The Tanner Lectures were delivered in Cambridge under the auspices of Clare Hall, 30 April–2 May 1984.

There is a third scenario. For example, at the end of the Cretaceous the Dinosaurs became extinct, whereas the mammals survived and radiated. I do not know why this was so, but let us suppose that the mammals survived because they are homoiotherms, and that their ability to chew had nothing to do with it. It would still be true that the Cretaceous extinctions led to an increase in the proportion of species with secondary palates, but not because of any selection for the palate itself. This is what Elizabeth Vrba has called the 'effect hypothesis'. It is an analogue, at the level of organs, of the process of 'hitch-hiking' at the level of genes.

The hierarchical view of evolution, then, is that processes of selection and stochastic drift go on at the level of genes and of species, as well as of individuals. As an old-fashioned proponent of the modern synthesis, I have no difficulty in accepting this, so long as no one expects me to believe that adaptations of individuals can be explained by molecular drive, and so long as the concept of species selection is confined to qualities, such as speciation rate and evolutionary rate, that are properties of species and not of individuals. However, there remains plenty of room for disagreement about the relative importance of these various levels, or — what amounts to the same thing — about the relative effectiveness of selection at different levels.

In from the cold

This brings me to what I see as the greatest impact that palaeontology is having on the way we see the mechanisms of evolution. We have been familiar for a long time with the dramatic disappearance of the Dinosaurs at the end of the Cretaceous. It is now apparent that massive extinctions, involving many different taxa, have been a repeated feature of evolution. Adolf Seilacher (Tubingen University) and Anthony Hallam (Birmingham University), the two palaeontologists on the panel, agreed with Gould on this, although there was disagreement about whether these events have been periodic or irregular, and whether they are caused by extraterrestrial events (meteorites, asteroids) or by terrestrial ones (Hallam, for example, emphasizes the role of changes in the area of the continental shelf caused by continental drift — see *Nature* **308**, 686; 1984). The impact of these extinctions is not random; in any given event, some taxa are more affected than others: Seilacher stressed the need for a quantitative study of this, to replace the somewhat anecdotal picture we now have.

In addition to the problem of their causation (which at present seems to be a problem for geologists and astronomers, although Seilacher did not rule out the possibility that some extinctions had biotic causes), these extinctions raise questions for evolutionary biologists. Is it possible that evolutionary change would slow down and stop in the absence of changes in the physical environment? As Manfred Eigen has pointed out, the simplest evolving systems (populations of RNA molecules in test tubes) reach a global optimum and then stop. Are extinctions, then, a necessary motive force of evolution? A second question concerns the relation between extinction and radiation. Ecologists tend to see nature as dominated by competition. They would therefore expect the extinction of one species, or group of species, to be caused by competition from another taxon. Most palaeontologists read the fossil record differently. The Dinosaurs, they believe, became extinct for reasons that had little to do with competition from the mammals. Only subsequently did the mammals, which had been around for as long as the Dinosaurs, radiate to fill the empty space. The same general pattern, they think, has held for other major taxonomic replacements. Not all palaeontologists would agree, but I think this is the majority view. I find it surprising: I would have expected a major cause of extinction to be competition from other taxa.

The Tanner lectures were an entertaining and stimulating occasion. The palaeontologists have too long been missing from the high table. Welcome back. □

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Malarial immunity

Indonesian and Sudanese style

from F.E.G. Cox

RECOVERY from malaria is accompanied by the production of antibodies that block the entry of merozoites — the infective stage of the *Plasmodium* parasite — into red blood cells[1]. Alternatively, it can be accompanied by a mechanism that kills the developing parasites within the red blood cell. This phenomenon has been known since 1944, when the Taliaferros noted the appearance of disintegrating forms, 'crisis forms', of a simian malaria parasite, *Plasmodium brasilianum*, in the red cells of recovering monkeys[2], but received little attention until it became clear that crisis forms are associated with recovery from a number of different *Plasmodium* infections in laboratory animals[3]. Although crisis forms are not apparent in the peripheral blood of patients with malignant tertian malaria caused by *Plasmodium falciparum* (because the developing stages of the parasite disappear from the circulating blood), they can regularly be detected *in vitro* both visually and by a test in which the incorporation of radiolabelled hypoxanthine into growing parasites within the red cell is measured[4]. That test has recently enabled James Jensen and his colleagues to show that in the Sudan, and probably elsewhere, intraerythrocyte killing predominates over merozoite blocking as a means of defense against the parasite[5-7]. These observations should completely alter our concepts of the development of vaccines against the blood-borne forms of *Plasmodium falciparum*.

Jensen and his colleagues collected sera from some 300 people living in three regions of the Sudan and tested their ability both to block the invasion of red cells by merozoites and to induce crisis forms in erythrocytes containing 'ring stage' parasites. The population could be divided into those who had experience of serious attacks of malaria, of mild malaria or no experience of malaria[6]. The clinical status could not be correlated with the presence of merozoite-blocking antibodies but was correlated with the ability of the serum to induce crisis forms. The greatest number of crisis forms was induced by sera from the group with no experience of malaria and hence with the greatest immunity to the disease. The serum factor that causes parasites to die in the red blood cells is not antibody because antibodies on the merozoites are sloughed off as the parasites enter the red cells; moreover, activity is not lost with the removal of immunoglobulin[5]. Jensen and his colleagues call the factor 'crisis-form factor' (CFF)[6].

In the Sudan, then, clinical immunity to malaria can be correlated with the presence of CFF in the serum. But what of other parts of the world? Jensen *et al.* have now shown that Indonesian sera do not induce crisis forms *in vitro*[7]. In order to make a direct comparison, the earlier Sudanese studies were repeated with sera from an area where the prevalence of malaria was similar to that in Flores, where the Indonesian study was carried out. Overall, Sudanese sera have abundant CFF and little merozoite-blocking antibody whereas Indonesian sera lack CFF, and their anti-parasitic activity, where present, is associated with merozoite-blocking antibody. This indicates that genetic differences may be important in determining whether antibody or non-antibody factors predominate in immunity to human malaria.

These experiments are of great importance and should significantly alter our concepts of an antimalarial vaccine. It is conventional to identify and isolate blood-stage antigens of the parasite by means of monoclonal antibodies and immune sera[8]. Such techniques may well lead to a vaccine that elicits a high and specific antibody response. But will this be protective when sera with antibody titres greater than 1:10,000 have little merozoite-blocking effect[7]? And if merozoite-blocking is of

relatively little importance in natural immunity in Sudan, could it be the basis of an effective vaccine there? If genetic differences between the populations at risk have to be taken into account in malaria vaccine development, it will greatly complicate the task.

What is CFF? The short answer is that we do not know. As long ago as 1953, Trager and McGhee identified a factor in the sera of birds immune to malaria that inhibited infection by Rous sarcoma virus[9]. From this has arisen the possibility that CFF is related to the ill-defined tumour necrosis factor[10,11]. Alternatively, it may be related to certain reactive oxygen intermediates that bring about the occurrence of crisis forms both *in vivo* and *in vitro*[12,13]. However reactive oxygen intermediates are probably too short-lived to produce directly the effects recorded by Jensen *et al.*, although they could be involved in reactions with lipids to produce toxic substances such as aldehydes[14].

What must happen now is a switch of attention away from *Plasmodium* antigens that elicit the production of antibodies to those that elicit CFF. Since CFF is probably a product of macrophages, these cells and the T cells that activate them require much more attention. At a very basic level, however, we now know that CFF kills malaria parasites and thus, if characterized, could be used to treat the rapidly increasing number of drug-resistant cases for which there is no obvious alternative treatment. Further studies of CFF might also provide clues to the development of new antimalarial drugs. □

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100 years ago

THE *Bolletino* of the Italian Geographical Society for May contains a brief account of Signor Maurizio Buonfanti's late expedition across North Africa. After some trips to Yakoba and other little-known parts of Sokoto, he made his way through Gando to the Niger at Say, about midway between Timbuktu and the Binue confluence. Here he turned north, and for the first time *ascended* the Niger as far as Timbuktu. This feat, hitherto supposed to be impossible, was performed *in the dry season*, and the problem thus successfully solved possesses considerable geographical and commercial importance in connection with the attempts now being made to establish regular lines of water communication between Western and Central Sudan and the Gulf of Guinea. From *Nature* **30**, 131, 5 June 1884.

Astronomy

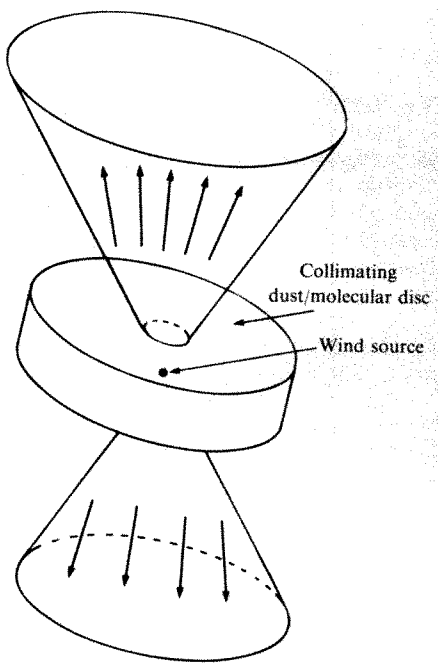
High-velocity winds in Orion

from Ben Zuckerman

THE biggest surprise to emerge from a decade of observational study of the formation of stars has been the realization that this process is accompanied by massive outflowing winds. The winds, which can be sporadic, are millions of times stronger than the solar wind and are generated by most, perhaps all, young stars either shortly before or, more probably, shortly after they initiate the burning of hydrogen into helium in their interior. Since it is natural to think of star formation as a process of collapse, or infall, the ubiquitous occurrence of an accompanying outflow comes as a bit of a shock. Now D.J. Axon and K. Taylor have described new optical spectra indicating very high-velocity winds in the most famous region of star formation in the heavens, the Orion Nebula (*Mon. Not. R. astr. Soc.* **207**, 241; 1984). Their spectra point towards the existence of a biconical pattern of outflow and suggest the presence of a substantial disc of molecular gas and dust grains close to a putative stellar source of excitation, located in the giant molecular cloud behind the nebula.

As a result of isolating the 6,300 Å wavelength forbidden transition of neutral oxygen, which is prominent in shock-excited regions but relatively weak in photoionized gaseous nebula such as the Orion Nebula, Axon and Taylor discovered a 'family' of six shock-excited objects which, in the plane of the sky, are located near a group of strong infrared-continuum sources discovered in the 1960s. The latter are believed to constitute a very young, but highly obscured, star cluster behind the Orion Nebula. The oxygen atoms display a very wide range of line-of-sight velocities extending from near 0 km s^{-1} (the velocity of the molecular cloud) to an approach velocity of nearly 400 km s^{-1}. When projection and other effects are taken into account, the implied wind velocities are of the order of 1,000 km s^{-1} — similar to that of winds from the most massive main-sequence stars in our Galaxy.

Taking into account previous observations of Orion, primarily at infrared and microwave frequencies, Axon and Taylor conclude that the most plausible model of the region is one in which a biconical protostellar wind originates from one (or more) of the massive young stars that are embedded in the molecular cloud, as in the figure. Although the star itself must be obscured from our view by an optically thick disc that lies, presumably, in its equatorial plane, the wind can escape from the polar regions. In their model, the shock-excited objects, detected by neutral oxygen emissions, are the result of the impact between the winds and gas blobs ('knots') located at the front face of the molecular cloud, just behind the Orion Nebula itself.



Model of biconical protostellar wind in the Orion Nebula. (From Mon. Not. R. astr. Soc. **207**, 246; 1984.)

These results bear on more general questions related to star formation and massive winds. Observations of many other regions of star formation indicate that bipolar or biconical outflow is the rule rather than the exception. Orion has now been reasonably securely added to this class. If the collimation is due to a disc of gas and dust located near the stars, as Axon and Taylor, like many others before them, suggest, then it is possible that at least the raw material necessary to make planets can be found in the vicinity of most young stars. Since not a single planet has been identified with certainty outside our Solar System, the implied ubiquitous existence of this raw material is of considerable interest.

Material which may be distributed in the form of a small disc with dimensions only about three times that our Solar System has very recently been seen directly (via scattered light at 2 μm wavelength) near the very young star, HL Tauri, by two different teams (S. Beckwith, B. Zuckerman, M. Skrutskie and H.M. Dyck, and S. Strom and G. Grasdalen, *Astrophys. J.*, in the press). However, it still remains to be shown how frequently a gaseous disc, as opposed to some other physical mechanism such as a magnetic field, is actually responsible for collimation of the winds that accompany star formation. □

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Protein structure

From *Bacillus* to rabbit

from Roger H. Pain

ON page 467 of this issue, Poorman *et al.* suggest a three-dimensional structure, complete with binding sites for substrates, activators and inhibitors, for a mammalian multisubunit enzyme, largely on the basis of the amino acid sequence of the enzyme and the crystallographic structure of the equivalent, but smaller, enzyme from a bacterium. Their report is of intrinsic interest to the protein chemist since it suggests a model which is testable by experiment. It is also of broader interest because it illustrates both the confidence with which biologists now assert that protein conformation is selected for and preserved during evolutionary processes and the way in which speculation on protein structure can be in order.

The enzyme is phosphofructokinase (PFK). It operates in the glycolytic pathway which is the means, common to all living organisms, of abstracting useful energy from the oxidation of glucose. PFK is particularly important because its activity can be modulated to control the rate of oxidation of glucose in response to energy demand in the cell. The reaction catalysed is the phosphorylation of fructose 6-phosphate by ATP to yield fructose 1,6-diphosphate and ADP. Insofar as they are all activated by raised concentrations of ADP, the enzymes from all species behave similarly. The greater sophistication of higher organisms, however, is reflected by the fact that their PFK is also activated by fructose 1,6-diphosphate and is inhibited by ATP. Moreover, their PFK is inhibited by citrate, allowing feedback control from the citric acid cycle. The greater complexity of the eukaryotic enzyme, requiring additional specific binding sites, is reflected in a molecular weight for each of its four identical subunits that is approximately double that of the prokaryotic enzyme.

To date, the only three-dimensional structure of PFK to have been determined is that of the prokaryote, *Bacillus stearothermophilus*. The structure of a eukaryotic PFK, the rabbit enzyme being the most studied, is needed in order to understand the more sophisticated mechanisms by which it is controlled. Poorman and colleagues have attempted to provide this, based mainly on comparison of amino acid sequences. They have determined the sequence of rabbit PFK and found that the subunit sequence falls into two similar halves, with more than 30 per cent of their amino acids being identical. Comparison of the two halves with the sequence of *B. stearothermophilus* PFK shows that 34 per cent of the amino acids of the carboxy-terminal half, and 44 per cent of the amino-terminal half, of the rabbit enzyme are identical to those in the bacterial enzyme.

This is taken to signify that the eukaryotic enzyme is the result of a duplication of the prokaryotic gene, with subsequent independent mutation within certain constraints. A further unknown mechanism must account for the additional 30 amino acids which join the non-identical twin domains of the rabbit enzyme. By packing four such 'double-bacillus' subunits together, with due regard to symmetry and the location of ligand-binding sites, Poorman *et al.* have constructed their three-dimensional model of rabbit PFK.

By straightforward extrapolation from the bacterial enzyme, rabbit PFK would be expected to contain 8 substrate binding sites and 8 ADP effector sites. Sequence comparison has led to identification of the four fructose 6-phosphate binding sites that are known to exist in rabbit PFK; sequences for the other four are significantly altered by mutation and are postulated to be fructose 1,6-diphosphate activation sites. It is also assumed that four ADP effector sites have been mutated to form ATP inhibitory sites.

These arguments rest on two generalizations. First, that such close relationships between amino acid sequences are the result of the organisms being at points on an evolutionary tree separated by a divergent process. Second, it is assumed that despite appreciable changes in amino acid sequence and despite the fact that certain amino acid substitutions can lead to a dramatic destabilization of three-dimensional conformation, the two sequences will fold into similar conformations. The 'code' relating sequence to conformation is known to be degenerate. The assumption is that selection operates on the one hand to keep the changes in sequence within these limits of degeneracy so that conformation does not vary significantly and, on the other hand, to retain certain active sites and to allow others to be modified so as to lead to more advantageous catalytic activity. This emphasis on the conservation of conformation is intriguing and the reasons for it are by no means certain. It is fascinating to see how these generalizations, only relatively recently established by comparing known sequences and conformations, are now being used in reverse to predict conformations from sequence — in this instance, of rabbit PFK. □

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Bacterial motion

Progress in flagellation

from Michael Spencer

IT is now more than ten years since the electrifying announcement that bacterial flagella actually rotate rather than propagate helical waves like larger motile organelles. The discovery was all the more surprising because it was by then becoming clear that flagella could adopt a number of different waveforms by internal rearrangement of the flagellin monomers, and an extension of this to a mechanism of active wave propagation did not require any dramatic act of faith.

From that watershed in knowledge have flowed two quite separate streams of thought. From recent publications, however, it now looks as if these independent streams, which have followed their courses for so long without meeting, are on the point of converging. Prominent in one stream is the school of Sho Asakura, which for at least two decades has continued a series of elegant demonstrations of the polymorphic nature of flagellar structure. These studies have been complemented by the ingenious theoretical predictions of a structural engineer, C.R. Calladine, and have led to satisfying explanations of the transitions that enable bacteria to change direction. The other stream of research has concentrated on the morphology and dynamics of the basal body that constitutes the flagellar motor; this has involved groups led by Howard C. Berg and others. They have confirmed that the motor is powered not directly by ATP but by protons, so that an electrical potential or a *p*H gradient across the membrane is sufficient to generate rotation.

Interest has been growing recently in the short region linking the basal body to the flagellum; it is normally sharply curved (and called the 'hook') and helps the larger-scale helix of the flagellar filament to insert into the motor in a direction perpendicular to the cell wall. It may also have enough flexibility to allow flagella to group into bundles. The structure of isolated hooks was determined a few years ago by Wagenknecht *et al.* (*J. molec. Biol.* **151**, 439; 1981 and **162**, 69; 1982). Although the monomers form a tubular structure and are packed in a very similar way to those in flagella, there are deep grooves in the surface of the hook that could facilitate bending without unduly reducing the ability to transmit a torque.

Some interesting new findings have now been published by Kato, Okamoto and

Asakura (*J. molec. Biol.* **173**, 463; 1984). They studied a mutant hook, the 'polyhook', which is much longer than in the wild type and can adopt either a straight form or a helical one; in the latter the amplitude and pitch are much smaller than those of the flagellar filament. Kato *et al.* now have evidence for both left-handed and right-handed helices, and suggest that the hook undergoes transitions between helical senses in the same way as the flagellar filament.

Like many discoveries, this raises more questions than it answers. The first surprise is that the 'normal' form of the hook (at neutral *p*H) follows a right-handed helix, whereas the rest of the flagellum is left-handed in the species studied — *Escherichia coli* and *Salmonella typhimurium*. This means that when the motor starts up in its normal sense of rotation, which is counterclockwise when looking towards the base, the torque transmitted to the hook is such as to unwind its helix (readers who feel dizzy when trying to visualize this are referred to Fig. 1*a*). In these conditions flagella tend to flip into an alternative helix of opposite sense.

Kato *et al.* speculate that when the motor starts up, the resulting stress may transform the hook into the alternative left-handed type, which would then have the same sense as the main flagellar filament. This raises intriguing questions about what happens during 'tumbling' or 'twiddling', the process by which bacteria periodically change direction so as to move up a favourable gradient (for a review see Berg *Scient. Am.* **233**, 36; 1975). At the start of a tumble the direction of motor rotation reverses and bundles of flagella that previously rotated in phase become jammed; the stress induces a change of sense in the flagella which then fly apart (Macnab and Ornston *J. molec. Biol.* **112**, 1; 1977). If the hook is held in a left-handed form during normal motion, presumably it reverts to right-handed when the motor reverses. Is this an essential step in the transformation of the filament?

A second question raised by the new results is whether the hook transformation can be explained on the model proposed for flagella by Calladine (*J. theor. Biol.* **57**, 469; 1976 and *J. molec. Biol.* **118**, 457; 1978). This model presumes the existence of 'protofilaments' or strings of monomers which may (through cooperative changes in monomer conformation) adopt either a 'long' or a 'short' state. If the protofilaments run round the tubular structure at an angle to its axis, the existence of two classes of protofilament in the same flagellum will cause it to coil into a helix (Fig. 1*b*). The parameters of the helix depend on the relative population of the two states.

The model predicts that in going from one state to another the 'twist' of the protofilaments will change, and that between a left-handed and a right-handed helix the 'twist angle' changes sign. In flagella the twist angle varies between −3° and +8°, which does not demand a substantial change in monomer packing. In the polyhook, however, the observed helical waveforms (from which twist angle was obtained indirectly) would require a range of about −50° to +20°. Even more puzzling, the protofilaments appear to be identified not with the 11 rows of contiguous monomers that fulfil this function in the flagellar filament, but with a set of '16-start' helices joining monomers that are not in close contact with each other.

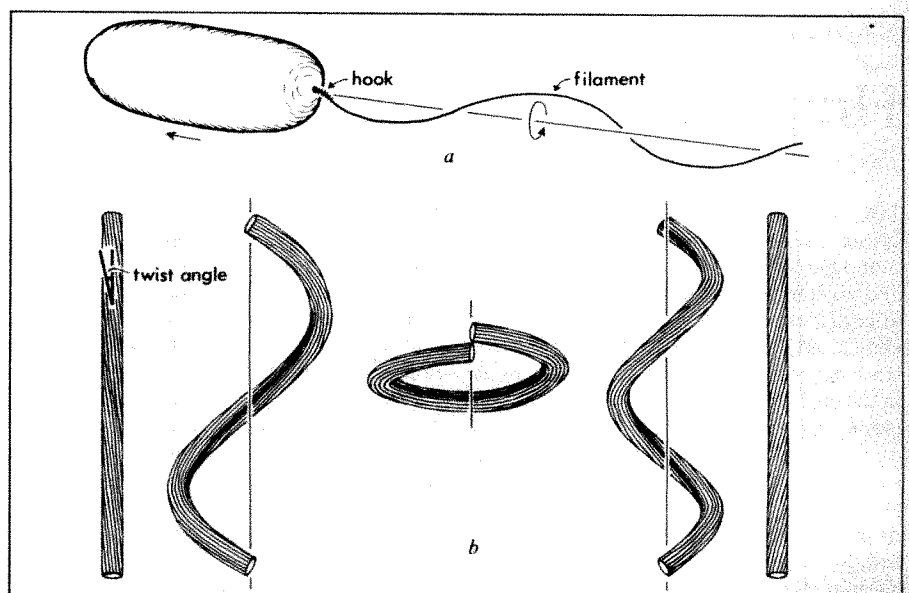


Fig. 1. *a*, Normal mode of forward propulsion by a flagellum for *E. coli* and *S. typhimurium*. *b*, Polymorphic forms of flagella obtainable by varying the twist angle (exaggerated here). The shortest protofilament (heavy line) always runs on the inside of the structure; in the two straight forms all protofilaments have the same length.

Kato *et al.* are suitably cautious about this conclusion, and mention the possibility that preparation for microscopy may have deformed the polyhooks; the matter will have to await direct observation of twist angles by high-resolution imaging of the surface lattice. A possibility not discussed in the paper is that in the hook the basic structure is invariable, as suggested by Wagenknecht *et al.* (see above), and that left-handed and right-handed forms involve protofilaments joining two different sets of points in the helical net. Neither of these needs necessarily be the set of 16-start helices discussed by the authors.

By a curious coincidence, a paper by Block and Berg on page 470 of this issue gives fresh significance to the old observation that certain components of the basal body 'motor' have a 16-fold symmetry. These authors have been studying a mutant of *E. coli* whose flagella are intact but paralysed unless the missing gene product is supplied. The appropriate gene under the control of the *lac* promoter was arranged to be carried into the bacteria as part of a transducing phage; the gene product was synthesized on addition of a *lac* inducer. When carried out with bacteria tethered to a glass surface by a single flagellum, the cells began to spin with increasing speed a few minutes after the addition of inducer.

The most interesting feature of the results is that the speed of rotation increased (or occasionally decreased) in discrete steps; at first there was a doubling of speed, which was followed by increases to speeds that were all multiples of the initial value for a given cell. The initial speed varied from cell to cell, presumably because of variations in the viscous drag on the cell body. Discrete steps could not be resolved beyond about the sixth, and most cells came off the glass or stuck to it before reaching their terminal speed. However, from one cell that continued rotating up to maximum speed and from other experiments on wild-type cells, Block and Berg calculate that the maximum number of steps is close to the magic number of 16. Their interpretation of this behaviour is that the gene product released by the *lac* inducer diffuses to the vicinity of the flagellar motor, which contains a number of independent force-generating units each requiring the gene product.

Block and Berg calculate that about 65 protons pass through a single force generator in one revolution, if the efficiency of the machine is unity — an assumption that may raise eyebrows among muscle biochemists. What might also attract their attention is that the torque per generator is calculated to be about 3×10^{-12} dyne cm; assuming that the generators act at a radius of 10 nm, one can infer that the tangential force per generator is about 20 times the average force generated by a muscle cross-bridge. In that sense bacteria seem to have a better design than muscle, and workers on flagella may have the more tractable problem. They still, however, share with devotees of muscle the need to find out how force is generated at the molecular level. □

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Neurology

Hopes of central repair

from Jeffrey S. Nye

THE fact that the adult central nervous system cannot be repaired after injury and that neurological deficits are permanent and unchanging was challenged recently at a conference* which brought together clinical and basic neuroscientists who are studying regenerative and repair processes in the central nervous system. The meeting made clear that clues from animal models of degenerative disease, as well as basic studies of regeneration in the nervous system, are increasing our understanding of neurodegenerative disease. Moreover, a radical change in the way some of the diseases are treated may be in the offing.

Controversy continues about the extent and importance of neurogenesis in adulthood [1]. Classically, only during embryonic life do neuronal precursor cells multiply. They then migrate from the ependymal or subependymal zones to their ultimate destination, and only there differentiate into neurones or glia. In fish and birds, however, the evidence for neurogenesis in adulthood is clear. Fernando Nottebohm (Rockefeller University) has shown that some avian species destroy and regenerate each year the neurones in certain forebrain nuclei [2]. In these nuclei, which subserve song production and perception, the acquisition and loss of neurones precede the bird's yearly acquisition and loss of song [3]. Thus neurogenesis serves an adaptive function in the life of songbirds. In the dentate gyrus and the olfactory bulb of adult rats, select populations of cells are born which are clearly neurones and not glia, in view of the presence of synapses and neurochemical markers [4,5].

Neuronal plasticity does not depend exclusively on the formation of new neurones. In primates, for example, all neurogenesis is complete soon after birth, but learning continues throughout adulthood. Pasko Rakic and colleagues (Yale University) argue that each neuronal population is born within its own discrete span of time during embryogenesis, some continuing into early infancy [6]. Despite extensive searching, no neurones born after this period have been found in primates, with the sole exception of the olfactory neurones, which are renewed throughout the lifetime of the organism [7]. Restoration of the capacity for neurogenesis in adult primates continues to be an attractive challenge to many neuroscientists.

Instead of growing new neurones, adult primates adapt by forming new synapses (synaptogenesis) and modifying old ones. What stimulates these adaptive processes remains an open question. In a model system using rats, Carl Cotman has shown that damage to neurones innervating the hippocampus will stimulate surviving neurones to increase the number of synapses they form with the hippocampus [8]. This reactive synaptogenesis may also be important for recovery of function after damage to neurones in aging and in neurodegenerative disease. In the aging rat, the rate of synaptogenesis after injury slows, but the same final number of synapses is reached as in younger animals. Quantitative similarity in the number of synapses, however, does not explain well known differences between the responses of old and young rats to brain damage. Indeed, Cotman has found decreases in the number of synapses contralateral to the injury in the hippocampus of old rats. He also finds differences in glial secretion of neuronal trophic factors in old versus young rats [9]. These findings may offer a possible mechanism for the poor recovery of old animals after injury.

A highlight of the meeting was the presentation by three groups of the startling results of their studies of neural tissue grafts. Albert Aquayo (Montreal General Hospital) and his group have been studying axonal growth after injury to the central nervous system [10]. In contrast to peripheral nerves, central neurones do not regrow axons past an injury; normally, they are prevented from doing so by astrocytes and oligodendroglia. However, when peripheral nerve sheaths, containing Schwann cells, are grafted next to the site of such an injury, some central neurones will extend axons as far as 3 cm through them. But when they reach the end of the peripheral nerve graft and re-enter damaged central nervous system, they stop growing and make no functional connections. Unfortunately, this impediment must be overcome before grafts of peripheral nerve can be used to repair damaged central nervous tissues.

Another approach to restore function after injury or in neurodegenerative disease requires grafts of neurones similar to those which are damaged or missing. Pioneering work has demonstrated that intracerebral grafts will establish reciprocal synapses with surrounding nervous tissue [11]. Functional recovery, however, requires that the grafted neurones establish a network of connectivity similar to that found in undamaged brain. The stringency of this requirement may depend on the site of damage. Might fetal cells, which are developmentally ready to send out processes and establish connections, retain these abilities outside their normal environment?

In a number of successful experiments, Lars Olson and colleagues (Karolinska Institute) have demonstrated that fetal neurones will survive grafting into the anterior chamber of the eye of a rat [12]. Moreover, neurones from the fetal substantia nigra implanted into the eye will generate the same firing pattern that they do *in situ*. When implanted adjacent to a fragment of striatum derived from an adult rat, fetal nigral cells innervate and establish functional connections with it.

In a similar series of experiments, the Lund group, led by Anders Bjorklund, has shown that injected fetal neurones from the septal nuclei will innervate a denervated hippocampus [13]. The implanted cells elaborate a pattern of cholinergic projections remarkably similar to that of the intact hippocampus. Even more astonishing, the grafts enable rats that have lost their cognitive abilities in a T-maze to regain them. Although the injected septal cells could not have received afferents from normal sources innervating the septum, the cells apparently possess enough intrinsic information to restore function to a denervated hippocampus.

Interestingly, Olson has also demonstrated that adult adrenal chromaffin cells can substitute for substantia nigra in innervating intraocular striatum [13]. In animal models, the implanted chromaffin cells begin to secrete dopamine, instead of their usual product, adrenaline. Both of the European groups have used dopaminergic cells from fetal substantia nigra to alleviate the motor deficits in animal models of parkinsonism. Furthermore, Olson has extended his studies of adrenal medullary implants, and shown that they too will provide enough dopamine to prevent some of the extrapyramidal symptoms of parkinsonian rats. For application to human disease, the use of adrenal grafts would avoid the ethical and immunological problems of using cells from fetal donors.

Olson's group has proceeded stereotactically to graft adrenal tissue from two patients with severe medically intractable

*The fourth annual conference of the Institute for Child Development entitled 'Hope for a new neurology' was held in New York City on 16–18 April.

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Parkinson's disease into the head of the caudate of the brain[14]. The success has been marginal. One patient showed no improvement (and no additional impairment) and the other has had some minor reduction of symptoms in the arms alone. Olson speculates that the caudate might not have been the right location for the graft to prevent motor symptoms; recent studies on monkeys have suggested that the putamen is the striatal site more closely correlated with motor function[15].

Despite these initial clinical failures, information from animal studies of neural grafting is accumulating rapidly. As work on the pathophysiology of Parkinson's and other neurodegenerative disorders progresses, investigators will test their new animal models with intracerebral grafts. It is likely that within a decade or two, neurologists will have another therapeutic option for severely disabled patients, and a new neurology. □

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Cosmology

Coloured comets

from David W. Hughes

WHAT colour is a comet? There is no easy answer. Visually, they are rarely seen in true colour because the retinal cones, which respond to colour, are usually swamped by the rods when it comes to the nocturnal vision of faint objects. George F. Chambers, a prolific cometary observer at the turn of the century, found that comets 'exhibit a more or less silvery-grey hue'. There were some exceptions. The sensational assertions of our mediaeval ancestors that comets had the colour of blood, or were fiery red, were discounted by Chambers, although tinges such as yellow, yellowish and ruddy were thought possible, particularly for bright comets.

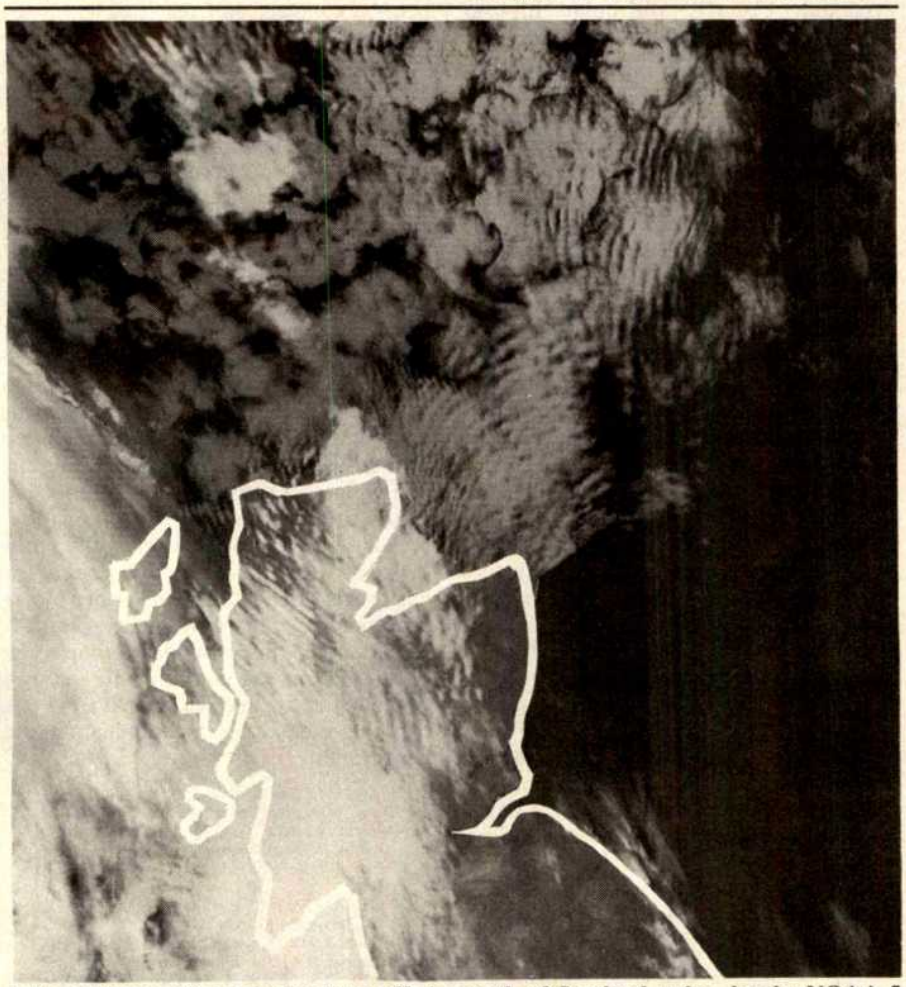
A quantitative approach to cometary colour can be made by measuring their brightness through a set of filters, as used recently by William Hartmann and Dale Cruickshank (*Icarus* **57**, 55; 1984). They analysed the V, J, H and K brightnesses of fourteen comets through filters centred on 0.55, 1.25, 1.58 and 2.2 μm. Defining an 'α index' as the position of a Solar System object on the J–H–K colour–colour diagram, Hartmann and Cruikshank find, crudely, that low (J–H), (H–K) brightness values (obtained by subtraction) are equivalent to high αs and vice versa. By looking at a range of asteroids, satellites and laboratory substances, the authors find that the α index increases both as a function of increasing albedo and as the ratio of ice to dirt area of the reflecting surface increases. The α index is also a function of colour, low values coming from dirtier redder surfaces, high values from bluer icier surfaces.

Data from the fourteen comets show clearly that cometary α indices increase as a function of heliocentric distance. Put simply, this means that as comets approach the Sun they become brighter and redden.

Unfortunately, the physical effects responsible for the α change are rather convoluted. First, changing the particle size distribution can change the colour, with the introduction of relatively more small particles causing reddening. Second, compositional changes can also affect the colour. Remote comets (such as Schwassman-Wachmann 1 at 6 AU) can release relatively stable icy-dust grains. When the comets are about 1 AU from the Sun, the ice component of the coma grain sublimates within less than an hour, so only the reddish carbonaceous dust remains to reflect sunlight. Notice that this mechanism could explain why the eruption of jets of fresh material from the nucleus can lead to sporadic colour changes — an injection of icier material turning the coma bluer. The third factor is phase. Asteroids certainly redden as they are observed at larger phase angles and comets could follow suit. Comets observed at similar phase angles do, however, exhibit significant differences in colour; the nature of the reflecting and scattering particles must therefore differ.

Hartmann and Cruikshank conclude that comets get redder as they approach the Sun and that this is not just due to a change of phase. The underlying cause is probably tied up with a decrease in the iciness of the dust being pushed away from the nucleus by momentum transfer from the sublimating gases. The closer a comet is to the Sun the hotter this dust becomes. Unfortunately we might have to wait for another brilliant daylight comet, the last of which was 1910 I, before we can see a blood-red comet with the naked eye. □

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Infrared image of cloud structures off the north of Scotland, taken by the NOAA 5 satellite at 0335 GMT on 23 March 1982. The wave-like features are caused by buoyancy oscillations — 'gravity waves' — occurring in Arctic or polar maritime air masses. From a recent paper in *Meteorological Magazine* (113, 97; 1984) in which K.J. Weston relates oscillations in wind speed and direction measured at the surface, with periods of about 20 minutes, to such high-altitude phenomena. Photograph: University of Dundee.

X-ray crystallography

Advantages of electronic 'film'

from Stephen C. Harrison

BY comparison with other areas of molecular biology, the determination of macromolecular structure has suffered because of the time-consuming and tedious course of X-ray crystallographic investigations. Recent advances in genetic manipulation have made it possible to produce considerable quantities of all sorts of interesting proteins and protein–nucleic acid complexes, some of which have been crystallized. It is therefore of more than casual interest that as a result of progress in the technology of X-ray detectors we appear to be on the verge of achieving a substantial acceleration in the collection of intensity data from crystals. The new detectors are two-dimensional position-sensitive devices which permit the simultaneous recording of large numbers of reflections with the accuracy usually associated with one-reflection-at-a-time diffractometers.

The earliest X-ray intensity measurements from crystals were made by following ionization with a gold-leaf electrometer. The hands of Bragg *père* and Bragg *fils*, actuated by the beat of a metronome, served as the 'stepping motors' for moving the crystal through its reflecting position. (Bragg junior wrote an entertaining account[1] of the times in which the intensity of the 100 reflection from NaCl could be reported[2] to be '676, when the crystal was turned through five minutes of arc for each beat of the clock'). As more complicated structures came to be studied, recording with photographic emulsions became standard, since it enabled many reflections to be measured simultaneously. The advent of automated diffractometers in the late 1950s turned a large part of the crystallographic community to electronic data collection, using a proportional counter or similar device, but the inefficiency of recording only one reflection at a time made diffractometry a poor choice for larger or more complicated proteins. A simple calculation presented by Arndt[3] shows that for unit cells larger than about 150 Å, appropriate photographic recording is the significantly more efficient approach. Indeed, stepped oscillation or rotation photography has become standard in most laboratories working on large proteins and at synchrotron installations supporting macromolecular crystallography.

Photographic film is actually an excllent detector, and for 1.5 Å X rays it is about 70 per cent quantum efficient. Automated two-dimensional densitometers, such as the widely used rotating-drum scanners, make digitization of films and evaluation of intensities relatively straightforward, although in a number of cases the tedium of this step has proved rate-limiting. A more significant disadvantage of photographic measurement is the limitation to its accuracy imposed by the small fraction of recording time during which a given reflection is diffracting. In oscillation photography, a 1° photograph is 'typical', but the angular range over which a particular order diffracts might be 0.1–0.2°, depending on geometrical factors and crystal quality. Background is recorded on the film during the remaining 0.8–0.9°, decreasing the signal-to-noise ratio. This disadvantage could be eliminated by the expedient of recording a series of 0.1° photographs — and it is precisely this possibility, impractical with actual film, that is offered by the new devices.

Currently there are three quite different two-dimensional detectors for single-crystal X-ray diffraction, each based on different design ideas and on significantly different technologies. The device developed by U. Arndt at the Medical Research Council Molecular Biology Laboratory in Cambridge, and now marketed by Enraf Nonius, records the diffraction pattern on a fluorescent screen connected by fibre optics to an image intensifier[4,5]. The intensifier output is read by a television system linked to a computer. The Gd_2O_2S input screen is 47 × 63 mm and the final scan is binned at 512 × 512 pixels. The size of the screen and the point-to-point resolution imply that data to 3 Å resolution can be collected at a single detector setting from crystals with a 100 Å cell constant. Crystals with larger unit cells require a longer crystal–detector distance and several detector settings. The device includes a 4-circle goniostat to facilitate resetting.

The detector developed by N. Xuong and colleagues at the University of California at San Diego is a multiwire proportional counter, derived from detectors developed for high-energy physics[6,7]. It consists of a xenon-filled ionization chamber with delay-line readout from planes of parallel wires in x and y. It has an active area of 27 × 30 cm, with a spatial resolution of 0.6 mm full width at half maximum (FWHM) in the horizontal direction and 2 mm in the vertical direction. The modest resolution necessitates a large crystal–detector distance (1 m or more), and full recording of a protein diffraction pattern to 3 Å resolution may require multiple settings or an array of more than one detector. The set-up in Xuong's laboratory has already been used to solve several structures, including dihydrofolate reductase[8] and the large ('Klenow') fragment of *Escherichia coli* DNA polymerase I (T. Steitz, personal communication). A similar device has been constructed at the University of Virginia.

A different type of xenon-filled detector has been developed by R. Burns of the Xentronics Corporation, Cambridge, Massachusetts. It is also a multiwire device, but with capacitive readout and with much higher spatial resolution (0.2 mm FWHM in both x and y). Its active area is 12 cm in diameter, and it is fitted with a modified oscillation 'camera'. The high spatial resolution permits a single detector to mimic a conventional X-ray film. The Xentronics detector has been used to collect data from several quite challenging crystals, including tomato bushy stunt virus, with a unit cell constant of 383 Å, and in our preliminary experience has shown very favourable intensity statistics[9].

Each of these devices is likely to have its own niche in the ecology of crystallographic applications. An advantage of the television-based design is its inherently higher data-rate capacity. The xenon-filled devices are at present limited by dead time to a total rate (over the face of the detector) of 30–50 kHz. Although not a significant disadvantage with conventional sources, this limitation can restrict possible applications with synchrotron sources. The Xenotronics device has the advantage of being a compact unit that can be moved easily from one X-ray set to another. Its large active area and high spatial resolution also give it an edge in the large-unit-cell range.

The gain in signal-to-noise that detectors offer with respect to film can be used either for increased accuracy or for increased speed. The increased accuracy will be especially helpful for medium-to-large unit cells, where reliable heavy-atom differences have sometimes been difficult to measure and noisy difference data difficult to interpret. The increased speed offered by these new approaches to data collection is likely to have immediate impact in the analysis of variants, mutants, modified proteins, active-site complexes and the like. Since these aspects of an investigation follow the initial structure determination, they are usually not held up by the phase problem (for example, the search for heavy-atom derivatives) but rather by the process of intensity measurement itself. It should soon be much simpler to couple site-specific mutagenesis and X-ray crystallography, in order effectively to test hypotheses based on the examination of a new structure. □

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Linear scale for acid rain?

SIR — "Acid rain" is a scientific issue for which careful presentation of results is of considerable importance, especially as the issue has entered the domain public debate in the United Kingdom. It is therefore right to ask whether we always present the results of acidity measurements of rainfall and freshwaters in the most appropriate way. The answer, I believe, may be no.

The acidity of rainfall and freshwater bodies is commonly reported in terms of the *p*H. This is a long established scale and one that is particularly convenient for expressing concentrations of hydrogen (H^+) ions (strictly speaking H^+ activity) when they range over several orders of magnitude. Difficulties in its use can arise from a failure to recognize that, being logarithmic, the *p*H scale is not linear. Confusion on this count, particularly in nonscientific circles is easy to imagine. Even in scientific circles a drop in *p*H of say 0.5 units appears at times to be treated as being equivalent, whether it occurs from *p*H 6.0 or from *p*H 5.0. In fact, the former case represents an increase in the H^+ ion concentration of some 2 microequivalents per litre, the latter of around 22 microequivalents per litre.

To give a more specific example, definition of trends over time in acidity levels has been commanding a great deal of attention. This is of some importance in establishing the relationship between emissions of sulphur dioxide and nitrogen oxides and subsequent acidification of the environment. Unfortunately, records of *p*H measurements of both rainfall and natural water bodies go back only several decades and even then there are problems over the continuity and quality of the data. Diatom stratigraphy in lake cores appears to provide one means of overcoming these problems, as demonstrated in the recent paper by Flower and Battarbee[1]. By measuring the changes in the diatom population in a ^{210}Pb dated sediment core they were able to reconstruct the *p*H history of a lake in the south-west of Scotland over the last 400 years or so (Fig. 1*a*).

It is at this point, however, that caution needs to be exercised. Interpreting trends on the basis of the data as presented in Fig. 1*a* is not straightforward, due to the use of *p*H on the abcissa. Expressing the results in terms of H^+ ion concentration on a linear scale gives a significantly different impression of the trend over time in this lake (Fig. 1*b*).

To emphasize this point, the results in Fig 1*a* indicate that about half the total change in *p*H had taken place by around 1900. Furthermore, almost all of the change had taken place by 1930 with very little change after 1940. In other words the major *p*H change took place early in the first half of the present century. But in terms of H^+ ion concentration, half of the change had taken place by the later date of around 1920, while there was a further steep increase from around 1950 to the present (Fig. 1*b*).

The use of a linear representation of concentrations provides the most appropriate means of representing the magnitude of any changes in concentration. (This is not to say though that effects are necessarily related linearly to concentration.) It is therefore encouraging to see a move to the use of a linear scale in reporting rainfall chemistry data in the United Kingdom[2]. Indeed a widespread adoption of the linear concentration scale when reporting H^+ ion concentrations, especially in acidic rainfall and freshwaters, would appear to be overdue.

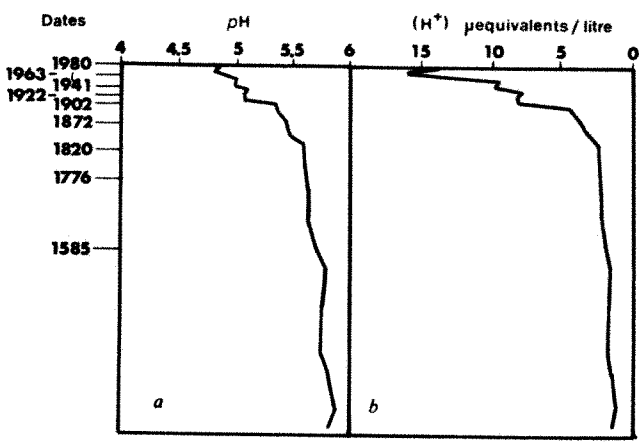


Fig. 1 Reconstruction of H^+ ion concentration history in Round Loch of Glenhead. *a*, Using *p*H scale (logarithmic); *b*, using linear concentration scale. (Based on ref. 1.)

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Alphabet and the Western mind

SIR — The unprecedented genius of Greece is unexplained. I conjecture that the modern study of the mind provides some tentative answers.

Before the Golden Age of Greece, the Greeks had developed their alphabet. This writing system is unique in possessing, by symbolizing vowels as well as consonants, a fully phonetic representation of language. This is achieved at the cost of limiting the representation of writing in the brain, as only the left cerebral hemisphere contains areas which are competent in the required phonetic analysis[1]. This has led to the ubiquity of the association, in the alphabet-dominated modern world, between injuries to the left hemisphere and impairments of writing and reading skills. This, however, is not a necessary restriction with all writing systems. Non-phonetic writing systems can use reading strategies involving the right hemisphere. Indeed, neurological studies show that hemispheric representation of non-alphabetic writing systems differs markedly from that encountered with alphabetic ones. Hatta[2,3] and others[4,5] have shown that logographic characters are more efficiently recognized by the right hemisphere. Sasanuma[6] has shown that *Kanji*, the non-phonetic writing system of Japanese, is vulnerable to different areas of brain injury from *Kana*, the phonetic system of Japanese writing.

There is evidence that reading retardation is associated with reduced involvement of the left hemisphere in reading[7]. In its absence such readers rely on the right hemisphere, which has been shown to have the ability to read simple English words independently of the left hemisphere[8]. In the absence of left-mediated phonetic analysis, the reading of frequent lexicals can occur through a non-phonetic strategy of logographically recognizing them from their shapes. It has been shown that retarded readers can learn to read Chinese logograms with their English translations more proficiently than their alphabetic equivalents[9]. Present studies of right hemispheric reading competence uses alphabetic lexicals which require left hemispheric phonetic decoding skills that disadvantage it rather than logographic ones which do not. I therefore suggest that the reading ability of right hemispheres for these and other non-Western writing systems will be found to have been underestimated. The right hemisphere, I conjecture, is fully competent, probably more so than the left hemisphere, to read, at the level of understanding which ancient societies used them, hieroglyphics, logograms and other non-alphabetic writing systems.

There are independent reasons for believing that pre-alphabetic writing systems were represented in the right hemi-

sphere. Such systems adopt a sinistral, that is, right to left, direction of writing and reading scan. Studies of eye scan direction have shown that sinistral scan direction favours a perception of lexicals to the left of the visual fixation point[9]. Such displacement of lexical perception into the left visual field would be expected if lexicals were preferably processed in the right hemisphere. Likewise as dextral scanning produces a perception of lexicals to the right of the fixation point, the dextrality of the Greek alphabet would follow from the adoption of a left hemispheric perception of lexicals through the right visual field.

Why should the development of a left rather than a right or bilateral representation of writing, as a consequence of the alphabet, have led to the Greek intellectual revolution? The answer probably lies in the fact that "competences" within the brain compete for expression. Analogously to Mendelian genes, the competences of the mind can have a dominant or recessive relationship to each other. Levy and Trevarthen[9] have shown that when two hemispheres each possess a competence able to answer a question, only one will do so. Though often the dominant competence will be superior in correctly replying compared with the recessive competence it dominates, frequently the recessive competence, as found by unilaterally testing it, will have a superior ability to answer to the question correctly than the competence which dominates it.

I conjecture that the alphabet, by producing a unilateral representation of lexicals in the left hemisphere, freed recessive competences in the left hemisphere which underlie rational, analytical and logical thought. These competences were previously overshadowed, inhibited and restricted in their development by a right hemispheric set of competences deriving from its representation for lexicals.

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Alignment of base sequences

SIR — In a recent paper Richards *et al.* show[1], in their Fig. 4, an alignment in which they claim that gaps "were inserted to maximize the number of base matches". The authors are to be commended on the clarity of phrase "number of base matches" when so many would have preferred the ambiguous term "homology". They have, nevertheless, still managed to mislead the careless reader as the following segment of their alignment shows:

```
------CCTTCAGAATACAGAATAGGGACATAGAGA
ATCCCACCCAGCCCCCTGGACCTGTAT---------
```

(Underlining shows the base matches.) An alternative alignment might be as follows:

```
CCTTCAGAATACAGAATAGGGACATAGAGA
ATCCCA---CCCAGCCCCCTGGACCTGTAT
```

This alignment reduces the number of gaps in this region by 50 per cent, reduces the number of gapped residues by 80 per cent, and increases the number of base matches by 133 per cent. More modest improvement around two of their other gaps is also possible. If I can increase the number of base matches by removing gaps, then clearly the authors' insertion of gaps did not maximize that number. One should seldom claim that one has maximized anything in the absence of a rigorous algorithm to accomplish that task[2]. It is sufficient (and frequently correct) to assert that gaps have been introduced to increase the number of base matches.

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Fissure animals of Bristol and South Wales

SIR — Michael Benton has given a fair account of the published material on the Upper Triassic and Lower Jurassic fissures of Bristol and South Wales[1]. However, in reviewing my work on the fissures, he missed some important aspects.

He states that "we must now view the fissure animals as representing a sample of a lowland fauna rather than a peculiar upland fauna". In particular, the tetrapods should be viewed as representing an *insular* lowland fauna and not the usual continental lowland fauna. The poorly dated mammal-bearing fissures of South Wales are generally described as being on islands[2,3] of (probably) Lower Liassic Age, whereas the specifically dated[4] Tytherington fissure fauna can be viewed as being derived from a small low-lying Rhaetian island habitat[5]. Indeed, the incidence of these tetrapod faunas, which are dominated by species with a small body size, is remarkably similar to Quaternary herpetofaunas found in solution fissures[6] or banana-holes[7] of modern-day islands.

The importance of this concept, that the Bristol and South Wales fissure animals are derived from insular Triassic/Liassic faunas, cannot be underestimated. Because of the small land areas on which they live each reptile species would have had a relatively low overall population size and thus would be subject to rapid rates of genetic drift and a high probability of extinction. Insular faunas are impoverished and show evidence of disharmony[8]. Therefore the biota could be expected to be unusual in respect to other preserved faunas of the same age, and any given fissure reptile species may show aberrant features compared to its contemporaneous unpreserved relatives. In this respect, rather than being closely related to *Anchisaurus* as generally proposed[9], *Thecodontosaurus* is more likely to be a dwarf plateosaurid (either *Plateosaurus* or *Lufengosaurus*). Full details are in preparation but, for example, it is the case that the basioccipital and basisphenoid of *Thecodontosaurus antiquus* discovered at Durdham Down is much more similar to that of *Plateosaurus* than that of *Anchisaurus*.

The high turnover rates expected on these small limestone islands lying near the Welsh and European landmass would also provide one alternative interpretation of the differences between the limestone faunas of the Slickstones fissures described by Fraser and Walkden[10].'

The remarkable preservation of the many tiny reptile bones can easily be explained by the short transport distances from death to burial expected in small islands. Indeed, deposition of the bones may be at least partly below sea level (see ref. 5).

Finally, reptile remains were described from Durdham Down fissure[11], and lepidosaur vertebrae were found in Holwell[12]; both these reports predate the description of *Clevosaurus* in 1939, that Benton refers to as the earliest description of reptile remains from these fissure deposits.

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Seminal lymphocytes, plasma and AIDS — Erratum

IN the piece of "Scientific Correspondence" by Keith James in the issue of 10 May (*Nature* **309**, 117; 1984) the reference list was omitted. It should read:

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Stoichiometry and the structure and stability of inorganic solids

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Stoichiometry (cation/anion ratio) is an important factor in determining the structure and stability of many inorganic crystals. It directly affects the coordination numbers of atoms, the relative stability of oxidation states and the tendency to form compounds with bonds between like atoms. Cation-rich binary compounds, especially oxides and nitrides, are either relatively unstable (have reduced bond energies) or do not exist. In such cases peroxides and azides are preferentially formed; these have normal bond energies.

WE are concerned here, in a very general way, with the role of stoichiometry in determining the structure and stability of simple inorganic solids. Although elementary, its importance in chemistry has generally been underestimated (but see ref. 1). We find that several observations, otherwise not readily explicable, are susceptible to a simple rationalization in terms of the constraints imposed by stoichiometry (which are ultimately those of valence). The emphasisis is mainly, but not exclusively, on binary compounds of main group metals with nitrogen, oxygen and fluorine.

We first distinguish between normal compounds and polycompounds. The former are those in which there are bonds only between unlike atoms (as in SiO_2). In the latter[2] there are also identifiable bonds between like atoms (as in azides and peroxides). These terms provide at best a rough classification; in general there is no rigorous criterion for deciding whether any two atoms in a molecule or a crystal are, or are not, bonded together. It is well known[3,4], for example, that bond-order criteria based on interatomic distances (d) often lead to results contrary to intuition. (For example, in Mg metal, d(Mg–Mg) = 3.21 Å, whereas in MgO, d(Mg–Mg) = 2.98 Å.) Equally, it is now recognized[5] that the presence of a build-up of electron density between atoms does not automatically indicate the presence of covalent bonding.

In the normal compounds of the main-group elements, the oxidation states are mainly determined by the position of the element in the Periodic Table. Accordingly, there is usually just one normal binary composition for a given pair of elements (such as, Cs_2O), although the compound may well be polymorphic. (There are obvious exceptions involving mainly pairs of the more-electronegative elements such as the sulphur or nitrogen oxides.) On the other hand, the same pair of elements may well form a number of polycompounds such as Cs_3O, Cs_2O_3, $Cs_{11}O_3$ and Cs_7O. Note that Cs_2O_3 [$= Cs_4(O_2)_3$] has both polycations and polyanions.

Pairs of elements which form stable normal compounds, very often do not form polycompounds [for example, AlN but not $Al(N_3)_3$] and conversely, those which form stable polycompounds often do not form stable normal compounds (for example, NaN_3 but not Na_3N). In what follows we generally discuss stability with reference to dissociation to free atoms (bond energies); although by this test Na_3N may well be stable, however, we shall argue that in this instance the bond energy is unusually low.

Our approach is largely phenomenological. In particular we will not discuss whether compounds are 'ionic' or 'covalent', although for convenience we shall use the terms *anion* and *cation* when the context leaves no room for ambiguity. We shall, however, offer an interpretation of our observations on the properties of metal-rich compounds (those with cation/anion stoichiometric ratios significantly greater than one) in which we identify a destabilizing influence of cation–cation interactions. Here a 'size' effect clearly emerges.

Bond energies and strengths

The term bond strength is used as defined by Zachariasen[6], so that for bond strengths v_{ij} between atom i and neighbours j

$$\sum_j v_{ij} = z_i \qquad (1)$$

where z_i is the valence of i. For a symmetrical coordination polyhedron v is the Pauling[7] electrostatic valence. In the absence of large interactions between next-nearest neighbours, v_{ij} is, to a good approximation, a unique function of the bond length r_{ij}.

Elsewhere[8] we have used the concept of equivalent bond enthalpy (the bond dissociation energy per unit bond strength) and demonstrated that (with exceptions of special interest below) it is approximately constant for bonds to a given metal in oxides. The enormous number of natural chemical compounds shows that equivalent bond enthalpies must be approximately constant for any pair of elements.

In rare instances, for example CO_2, high order ($v > 1$) bonds are favoured. More commonly, the situation is one in which structures with many weaker bonds are favoured to an alternative with fewer strong bonds: an example is SiO_2. Thus, in general, large coordination numbers are preferred, and this gives rise, for most combinations of elements, to solid structures. Fragmentation of structures into molecules, with low coordination numbers, is the exception, and occurs only with relatively few combinations of elements. We are mainly concerned, therefore, with the factors that provide upper limits to coordination numbers.

Coordination numbers in crystals

In most crystals, coordination numbers are determined by three principal factors. The first two involve a balance between the tendency to form as many bonds as possible, and the interactions (normally, but not necessarily, repulsions) between the next near-nearest-neighbour atoms. Note that when considering such interactions, the 'size' of an atom is its non-bonded size[9], which has no relationship to conventional ionic radii. Indeed, the failure of the ionic radius ratio rules for coordination numbers has long been recognized[10], but still not generally accepted. This neglect has led to the erroneous point of view that (to take a specific example) cation coordination numbers in oxides are

solely determined by oxygen–oxygen repulsions and not by cation–cation repulsions.

An important point is that cation and anion coordination numbers are not independent: by definition, each bond has a cation at one end and an anion at the other, so that the ratio of the average coordination number of the anions to that of the cations is equal to the stoichiometric cation/anion ratio[11]. Thus is SiO_2, ^{iv}Si implies—at least on average—^{ii}O, and we may ask whether the coordination number of Si in SiO_2 is limited to four (at ordinary pressure) because no more than four oxygen atoms can fit around a silicon atom, or because no more than two silicon atoms can fit around an oxygen atom. We prefer the second point of view[9].

In order that the shortest interatomic distances in normal crystals be between cations and anions, coordination numbers must clearly be less than, or equal to, 12. In binary compounds the limiting value is only reached in exceptional structures such as those of Cr_3Si and WAl_{12} (hardly normal compounds in the present context). In practice this third constraint limits coordination numbers in normal compounds of nitrogen, oxygen and fluorine so that values in excess of nine are very rare. It follows that when the cation/anion ratio is very different from unity, the majority component will have a rather low coordination number (thus four for Rb in Rb_2O and two and three for Li in Li_3N). This important influence of stoichiometry in influencing coordination numbers appears largely to have gone unrecognized although N. V. Belov (quoted by Lebedev[10]) apparently realized, but did not explain, the phenomenon.

Examples of unusual coordination numbers are to be found among the many cation-rich ternary oxide structures determined by Hoppe and coworkers[12]. Thus transition metal cations in planar 3-fold coordination are found in Na_4MO_3 (M = Zn, Fe, Co and Ni) and in $Na_{10}Co_4O_9$, and Ni(II) is in linear 2-fold coordination in K_2NiO_2. We emphasize that these low coordination numbers for the cations are due in large part to the high stoichiometric ratio of cations to anions in the compounds.

We have mentioned that in SiO_2, the coordination number of Si was largely determined by the difficulty of fittihg three Si atoms around an oxygen atom. This is neatly illustrated by the structure of SiP_2O_7 in which the increased anion/cation ratio allows silicon to be six coordinated by oxygen while keeping the oxygen coordination at two (that is $^{vi}Si^{iv}P_2{}^{ii}O_7$). There are several other silicon phosphates in which the coordination number of silicon is similarly controlled by stoichiometry[13].

Irregular coordination figures

The occurrence of irregular coordination figures in structures can also be a simple and inevitable consequence of stoichiometry. We illustrate this point by stoichiometry M_2X_3. If M is to have coordination number four, X atoms must exist in at least two different coordinations (the average coordination number of X being 8/3). Thus $^{iv}B_2{}^{ii}O^{iii}O_2$ for the structure of high-pressure B_2O_3. In this compound the sum rule, equation (1), requires that the strengths of the bonds to the two-coordinated oxygen are $v = 1$ and for the three-coordinated oxygen, $v = 2/3$. As bond strengths and bond lengths are strongly correlated[14] these two bond strengths correspond to different bond lengths—in this example 1.38 Å and 1.51 Å respectively. The coordination figure about boron ($B^{ii}O^{iii}O_3$) is correspondingly an irregular tetrahedron. The irregular coordination figure about La in A-type La_2O_3 (in which La is in 7-fold coordination) is similarly explained.

In such structures, the Pauling[7] electrostatic valence sum rule is not obeyed, and according to Pauling's hypothesis they are destabilized. Certainly there are no stable oxides of stoichiometry M_2O_3 in which all the M atoms are in four-coordination despite the fact that atoms such as B, Al, Ga, Fe(III) are frequently in four coordination in ternary oxides and such like. We caution against too much generalization; for some metals (notably the early transition elements) distorted coordination polyhedra are in fact more stable than regular ones (as illustrated by the structures of MoO_3 and WO_3). Nevertheless,

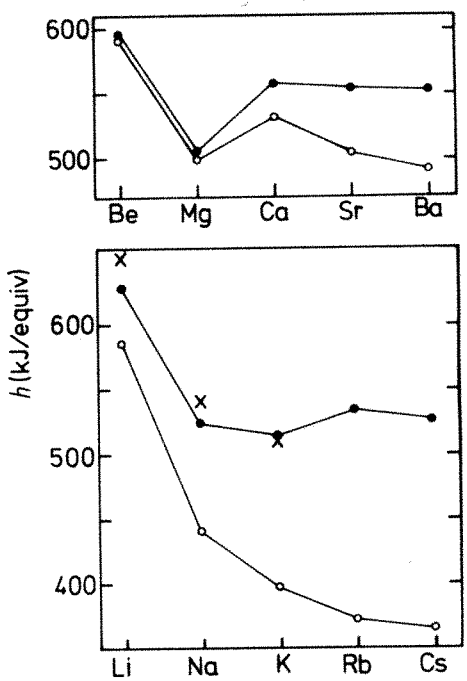


Fig. 1 The equivalent bond enthalpy, h, in alkali and alkaline earth oxides. ●, Ternary and similar oxides[8]; ○, normal binary oxides; ×, peroxides.

the instability of irregular coordination figures of the type just discussed is the reason for some departures from the general rule that cation coordination decreases with increasing cation/anion ratio. An obvious example is $^{iv}AlPO_4$ and $^{vi}Al_2O_3$; another is the structure[15] of Na_3NO_4 with ^{iv}N (nitrogen is considered a cation in this context) in contrast to ^{iii}N in $NaNO_3$.

Bond energies

An earlier study[8] of heats of atomization of oxides showed that there is a constant contribution, per unit of bond strength, for each M–O bond, to the total heat of atomization of ternary and similar oxides. With the exception of the alkali metals and, to a lesser extent, the heavier alkaline earth metals, the contribution from each element was equal to the equivalent heat of atomization of its binary oxide.

The data of that study (for Li, Na and K) have now been augmented with the few available data for ternary oxides of Rb and Cs (ref. 16). These have been used to calculate the equivalent bond enthalpies both in binary and in ternary and such like oxides that are shown in Fig. 1. Note that these quantities differ by an amount that increases with increasing size (atomic number) of the metal atom. The most obvious way that the binary oxides differ from the rest is in stoichiometry; the latter all had cation/anion ratios less than one, in the former it is two. We suggest that the higher proportion of metal atoms in the binary oxides result in significant cation–cation interactions which destabilize the crystal to an extent that increases with cation size. This view is strongly supported by the observation[17] that bond lengths in alkali metal oxides are anomalously lengthened (compared with those in ternary compounds) in a parallel manner.

Figure 1 also shows the M–O equivalent bond enthalpies in the peroxides M_2O_2. These were calculated from the heats of atomization of the solid peroxides by subtracting the O–O bond energy, the latter coming in turn from the heats of atomization of H_2O and H_2O_2 (ref. 18). It may be seen that they are normal in the sense that they lie very close to the points found for the ternary oxides, showing clearly that the increasing tendency to peroxide formation with increasing atomic number is due, not to enhanced stability of the peroxide, but rather to decreased stability of the normal binary oxides.

For the nitrides and azides fewer relevant data are available. However, Wattenberg's[19] work shows that if it exists at all, Na_3N

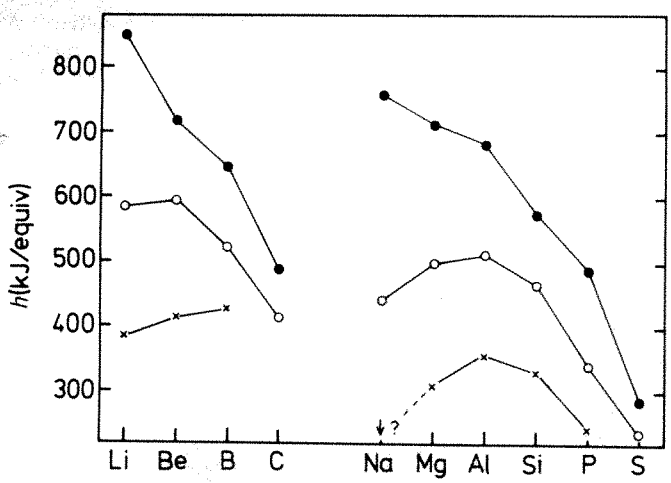


Fig. 2 Equivalent bond enthalpies (heats of atomization per equivalent) for binary solids. ●, Fluorides; ○, oxides; ×, nitrides. The arrow represents the upper limit for Na_3N derived in the text.

is metastable with respect to Na(metal) and N_2. Thus for the reaction $6Na + N_2 = 2Na_3N$, $\Delta G^0_{273} > 0$ from which we estimate $\Delta H^0_{273} > -57$ kJ mol^{-1}, and hence an upper limit for the equivalent bond enthalpy in the binary compound h_0(Na–N) < 260 kJ mol^{-1}. A value for the equivalent bond enthalpy h(Na–N) in NaN_3 may be obtained in the same manner as for the peroxides (that is using data for NaN_3, HN_3 and NH_3) giving h = 554 kJ mol^{-1}. Although rough estimates, the difference $h - h_0$ is striking and demonstrates that sodium nitride, like sodium oxide, has an anomalously low bond energy.

Figure 2 shows equivalent bond energies for normal binary compounds with N, O and F. The fluorides show the expected trend of bond energy increasing with increasing electronegativity difference. However, bond energies to N, and to a lesser extent, to O are clearly anomalous in the case of Na and, to a lesser extent Mg. (In contrast, the equivalent bond energies in ternary, and similar oxides[8] show the expected trend similar to that of the fluorides in Fig. 2).

Nonexistent compounds

We have called attention to the reduced stability (and consequent lengthened bonds) in certain metal-rich normal compounds. The effect was larger for heavier metal atoms and larger metal/non-metal ratio. It might then be expected that certain compositions are not realized as stable phases. This indeed occurs. It is also found that when the normal compounds do not exist, stable polycompounds are the rule. We are particularly concerned with providing a simple rationalization for the non-existence of normal binary compounds which might be expected to be stable on the basis of a large difference of electronegativity between the two components.

It is at first sight surprising that so few normal carbides of the main group elements are known. These seem to be restricted to SiC, Al_4C_3, Be_2C and, possibly[20], Li_4C. The other alkali metals readily form graphite intercalation compounds (a form of polycompound) and acetylides but not solid M_4C. The heavier alkaline earth metals form only acetylides. It seems clear that these observations may be simply and directly explained as a consequence of the large number of metal–metal interactions that would be imposed by the stoichiometry of the missing compounds. The non-existence of other compounds such as GeC may be ascribed to the same cause. Here we are noncommital about the nature of metal–metal 'interactions'; molecular orbital calculations[21] on molecules such as Li_4C and Li_3N show signs of a bonding overlap population between the lithium atoms and indeed hypervalent molecules such as Li_6C are also predicted[22] to be stable (and here there are clear indications of Li–Li bonding).

In contrast to the carbides, all the alkaline earth metals form normal silicides (increasing bond lengths act to mitigate metal–metal interactions). Silicon and aluminium do not react, but the difference in electronegativity is becoming rather small.

We have already remarked on the relative instability of Mg_3N_2 and by inference of the other alkaline earth nitrides, and of Li_3N. It is clear[18] that the other alkali metals do not form normal nitrides, but they all form stable azides. The size effect is also apparent here in that the rest of the normal alkali metal pnictides are well characterized[1].

The complementary instability of at least some polycompounds can again be ascribed to the constraints of stoichiometry[2]; $Al(N_3)_3$ for example, would be very crowded indeed—but now with anions rather than cations. The problem of the stability of peroxides and azides is closely analogous to that of the stability of oxysalts—for example, the only stable anhydrous perchlorate are those of the alkali metals and the alkaline earths other than beryllium[23]. Wells[1] has discussed the stability of such salts from a similar point of view to ours.

Oxidation states

We have presented quantitative evidence that cation-rich oxides have abnormally low stabilities. In the case of ternary transition metal oxides, it is clear that the cation/anion ratio could be decreased (and the stability increased) by increasing the oxidation state of the cation. Alternatively, a high oxidation state of a transition metal could be stabilized by forming a ternary oxide with a low valence metal, and, indeed, high oxidation states of transition metals are readily obtained in ternary alkali–metal oxides. Thus although the highest stable oxidation states in the binary oxides are Mn(IV) and Ni(II), $KMnO_4$ [with Mn(VII)] and Na_5NiO_4 (ref. 24) [with Ni(III)] are known.

Conversely, higher oxidation states are destabilized by a large anion/cation ratio. Despite the (frequently correct) assertion that fluorine tends to develop the highest valence of a cation [thus Ni(IV) in ternary fluorides[24]], this is not the case for transition metal cations having the potential of high valence. For example, CrO_3 is known but not CrF_6 (ref. 25). The oxidizing power of fluorine is allowed full rein when combined in ternary fluorides with alkali and alkaline earth metals (especially the heavier ones). Then we find, for example, Cs_2MF_6 with M = Cu(IV) or Ag(IV) [contrast M(II) as the highest oxidation state in the binaries] and $CsAuF_6$ with Au(V) (ref. 24).

Conclusions

We have shown how the constraints of stoichiometry affect both the structure and stability of simple inorganic solids. Although simple and obvious, our ideas provide a unifying understanding of a range of phenomena that have not previously been explained, except perhaps in isolated instances on an *ad hoc* basis. We have adduced only a few more-striking examples to illlustrate our thesis. But we have not omitted any counter-examples known to us.

There have, of course, been numerous previous attempts at developing useful generalizations concerning the relative stability of inorganic solids. One of the first and most successful was that of Ramberg[26], who compared the relative stabilities of pairs of salts, and deduced that the low-energy assemblages were those in which the most highly charged cations were combined with the most highly charged anions (that is, $Mg_3N_2 + LiCl$ rather than $Li_3N + MgCl_2$)—the interpretation of such rules in terms of the ideas we have set out here should be clear.

It should also be clear that we provide a rationalization for the observation that the heats of reaction between acidic oxides and basic oxides is large, for example,

$$Na_2O + SO_3 = Na_2SO_4 \qquad \Delta H^\circ = -579 \text{ kJ mol}^{-1}$$

whereas that between amphoteric oxides is small, for example,

$$MgO + Al_2O_3 = MgAl_2O_4 \qquad \Delta H^\circ = -25 \text{ kJ mol}^{-1}$$

However, note that many of the observations that we have made are not in accord with the generalizations of the theory of hard

and soft acids and bases (HSAB)[27] which has several features in common with Ramberg's treatment. Thus according to the HSAB approach, hard acids such as Na should form more stable compounds with hard bases such as N than with weak bases such as As. Our discussion shows that the converse is true. Another prediction[28] of the HSAB theory is that to stabilize an element in a high oxidation state it should be surrounded by hard bases such as O^{2-}. We have seen that in solid compounds, the addition of hard acids such as Na^{+} is, in fact, what is required.

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ARTICLES

Optical counterparts of unidentified IRAS point sources: infrared luminous galaxies

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Deep charge-coupled device imaging has been used to identify a number of IRAS (Infrared Astronomical Satellite) blank field sources with distant galaxies, and indicates that most high-latitude unidentified IRAS point sources have an extragalactic origin. The present sample of galaxies seems to include some with the largest known ratios of IR-to-optical luminosity, with values of L(IR)/L(B) *ranging upwards of 200. A redshift obtained for one source, 0422+009, indicates that this object has ~35 times the absolute IR luminosity of the starburst galaxy M82.*

HOUCK *ET AL.*[1] have recently reported several point-like 60-μm sources with no obvious visible counterparts. Possible explanations for the nature of these sources include high redshift galaxies, heavily obscured young stars, evolved stars with circumstellar dust shells, nearby 'Jupiter-like' bodies, or some fundamentally new type of object. To help establish which of the possibilities is correct, we have obtained deep, near-red images centred on the Houck *et al.* source positions.

CCD imaging

Houck *et al.*[1] present nine unidentified sources: six near RA 4 h, and three near RA 17 h. On the night of 9–10 December 1983, the prime focus charge-coupled device (CCD) camera of the 4-m telescope at Cerro Tololo was used to observe five of the RA 4 h fields. Four-minute *I* band exposures were obtained for each field, and for three of the sources, 5-min *R* band exposures were also secured.

Images of the observed fields are presented in Fig. 1. Following standard procedures[2], the CCD data have been trimmed, debiased, flattened to ~0.3% across the frame by exposures on both dome and smoothed blank sky, and corrected for fringes arising from night sky emission. Low-level (~1%) fringing remains in the data. Also shown in Fig. 1 are the IRAS error ellipses from Houck *et al.*[1], together with several galaxies that we have found lying within or near the ellipses. An examination of the radial brightness profile easily distinguishes non-stellar from stellar objects in our data. We first discuss each of the six RA 4 h sources.

0358+223. No CCD data were obtained for this field. However, a bright, stellar-like object is visible on the Palomar Observatory Sky Survey (POSS) at the virtual centre of the IRAS error ellipse[1]. Careful examination of POSS plates stored at Kitt Peak shows that the image of this object is 'soft', indicative of a distant galaxy which we identify with the IR source. The POSS prints examined by Houck *et al.*[1] are presumably inadequate for such fine discrimination.

0404+101. Figure 1 shows a faint galaxy near the centre of the IRAS error ellipse which we identify as the IR source. This object is visible just at the red plate limit of the POSS.

0412+085. We do not identify this source with any obvious optical counterpart. A faint galaxy does fall on the edge of the error ellipse, but we are hesitant to claim correspondence with it. First, because the IR-to-optical luminosity ratio would be uncomfortably large, even in comparison with the other great values derived later here; and more important, the probability of chance superposition for galaxies this faint becomes significant[3]. Further, re-examination of the IRAS data indicates that the source is probably extended, and therefore likely to be a hotspot on the IR cirrus (C. A. Beichmann, personal communication) present in this region (see ref. 4). The source has, in fact, the lowest signal-to-noise ratio of those reported by Houck *et al.*[1]. If it eventually does prove point-like, correspondence with

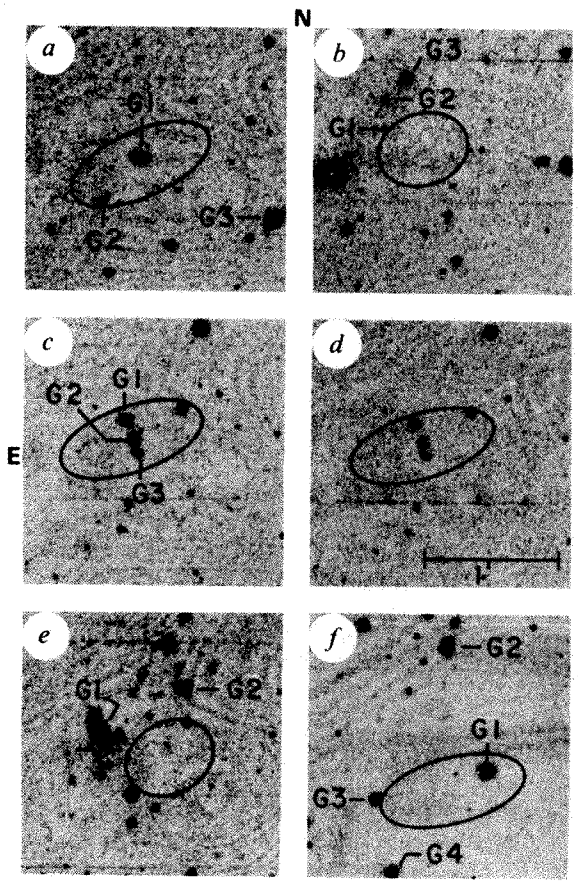


Fig. 1 CCD images of: *a*, 0404+101 (*R* band); *b*, 0412+085 (*I* band); *c*, 0413+122 (*R* band); *d*, 0413+122 (*I* band); *e*, 0422+009 (*R* band); and *f*, 0425−012 (*I* band). IRAS error ellipses from Houck *et al.*[1] are also shown. The alternative IRAS position for 0422+009 is marked in *e*. Galaxies are numbered as in Table 1. The orientation and scale for all images is identical.

galaxy number 3 may remain a possibility, although this object does fall a long way out of the present error ellipse.

0413+122. A galaxy triplet is located in the IRAS error ellipse. We suspect that galaxy number 2, near the centre of the ellipse, is the IR source. This object looks obviously disturbed, and the slightly distorted shape of galaxy number 3 is suggestive of tidal interaction between the two. The asymmetrical appearance of galaxy number 2 may be partially due to what looks like either a dust lane running along the north-east side of the object, or to two galaxies being viewed in close superposition. The triplet is again just visible at the POSS red-plate limit.

0422+009. Figure 1*e* shows that no obvious counterpart is present in the original IRAS error ellipse. However, an alternative source position obtained from later scans of the area (C. A. Beichmann, personal communication) places the IR source nearly coincident with a galaxy that appears to be the brightest member of a small cluster. Given the odd morphology and rather 'dusty' appearance of this object, we conclude that it is in fact the IR source (although there appears to be no *a priori* reason for preferring either of the two source positions marked in Fig. 1*e*).

0425−012. The radial profile of the stellar-like object in the error ellipse (see also ref. 1) establishes it as a galaxy, which we identify with the IR source. The image on the POSS red plate is also soft, although less obviously so than for 0358+223. (We have also examined the POSS plates at the positions of the three RA 17h sources listed by Houck *et al.*[1], and find that galaxies appear to be present in at least two of the error boxes.)

We now need to assess the probability of chance background contamination. For this purpose we use the r_F counts of Shanks *et al.*[3]; over the colour range of interest, $r_F \approx R$ magnitudes on

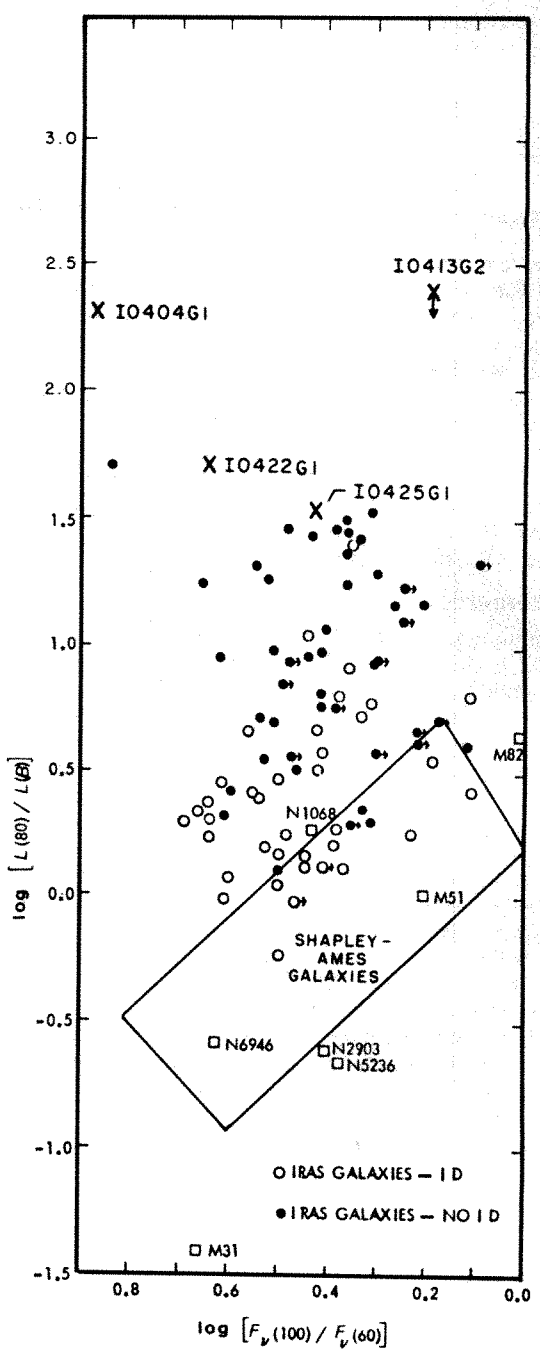


Fig. 2 The ratio of luminosity at 80 μm to that in the blue is plotted against the flux density ratio at 100–60 μm. ×, Galaxies studied here; ○, catalogued and ●, uncatalogued galaxies in the IRAS mini-survey[5]. The box encompasses the location of most Shapley–Ames galaxies[10]. Some selected nearby bright galaxies are also individually labelled.

the Kron–Cousins system, which we use for the photometry. Of the five IRAS sources we have identified, three have an estimated $R \leq 17.5$ mag, while two have an estimated $R \sim 19$ mag. For the typical error ellipse area of ~0.5 arc min^2, we find a probability of ~0.03 for random coincidence if $r_F \leq 17.5$ mag, which becomes ~0.16 for $r_F \leq 19$ mag. On an individual basis, then, the probability of chance background contamination is low; but, on the other hand, we can expect to find one interloper having $r_F \leq 19$ mag given six total surveyed fields. Hence, one of our suggested identifications may be spurious. This question can be further considered when more accurate IRAS source positions become available, but we believe that at present the source identifications given above are in most (and possibly all) cases correct.

We conclude that distant galaxies probably dominate the sample of unidentified point sources presented by Houck *et al.*[1], rather than galactic objects or some previously undiscovered type of source. Indeed, we have located galaxian counterparts for all five of the (remaining) RA 4 h Houck *et al.*[1] point sources.

Table 1 shows positions accurate to ~1 arc s for the four source identifications having new imaging data. These were measured from our CCD frames in relation to secondary positional standards, whose coordinates were in turn determined relative to nearby Smithsonian Astrophysical Observatory (SAO) Catalogue stars on the POSS plates. Figure 1 shows that correspondence with the Houck *et al.*[1] positions is generally reasonable (the one exception being 0422+009). In the lower part of Table 1, we give positions for other galaxies marked in Fig. 1.

The four galaxy identifications in Fig. 1 all seem to be members of small clusters, groups, or pairs, and at least two of these objects seem morphologically peculiar and may be interacting with nearby neighbours. This could be an important clue for understanding the extreme IR properties of these spirals. Interestingly, a high fraction of interacting neighbours was also found for the "IR galaxies" studied by Soifer *et al.*[5].

Photometry

RI photometry (on the Kron–Cousins system) is presented in Table 1 both for the source identifications and for the nearby companions. The photoelectric standardization again followed common procedures[2]. Magnitudes for both the standard stars and program objects were measured using an aperture photometer program at Dominion Astrophysical Observatory, and the estimated photometric accuracy of the data is~0.05 mag. The galaxy magnitudes quoted were found by first calculating brightness within successively larger apertures until either a stable magnitude was reached, or interfering objects were encountered. The latter was a problem only for 0413+122, and for all but the galaxies in this field, the magnitudes in Table 1 can be considered indicative of total magnitudes.

The $R-I$ colours of the source identifications, corrected for galactic reddening, range from 0.70 to 0.76. (Reddening corrections have been calculated using van de Hulst curve number 15 interpolated to the effective wavelengths of our filters; and for galactic extinction we adopt the colour excesses listed by Houck *et al.*[1].) Comparison with the $R-I$ colours of nearby disk galaxies is hampered by the fact that such photometry appears only rarely[6–8], and what does exist must be converted with some uncertainty from the Johnson to Kron–Cousins system[9]. Nevertheless, it seems that the $R-I$ colours of our IRAS source counterparts are completely normal. For example, the transformed spiral colours measured by Johnson[6] range from 0.66 to 0.78. The *RI* colours for the other objects listed in the lower part of Table 2 also do not appear unusual, except perhaps for the rather red colour of galaxy number 3 in the 0404+101 field.

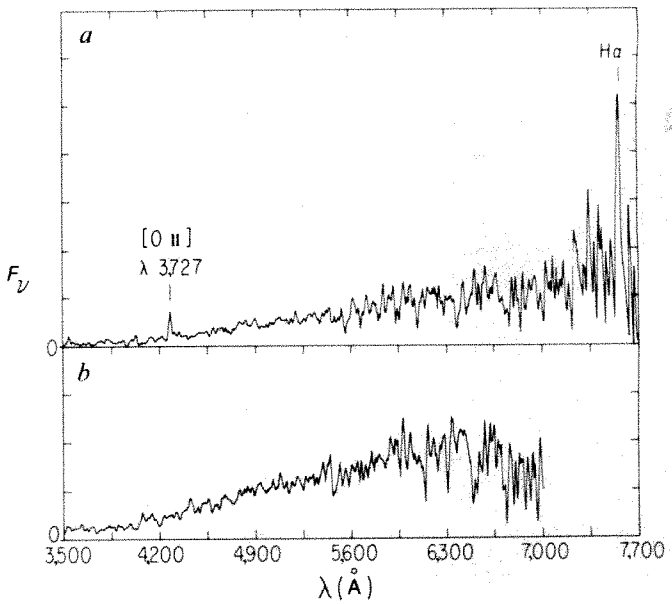


Fig. 3 MMT Reticon spectra of *a*, 0422+009 and *b*, 0425−012. The flux scale is linear. The spectrum in *b* has not been plotted longward of 7,000 Å, where it is severely degraded by noise.

The $R-I$ colour primarily measures the old giant population common to all galaxies, and apparently has little sensitivity to other galaxian components. In particular, Johnson's $R-I$ colour of 0.66 for the proto-typical starburst galaxy M82 falls within the range of colours covered both by his spirals and ellipticals. (Note also the *k*-correction for $R-I$ remains <0.05 mag for $z<0.2$; M. Lebofsky, personal communication.) Hence, the *RI* colours provide little insight into the physical nature of the IR sources.

With the present data we can, however, estimate the ratio of luminous output at 80 μm to that in the blue for comparison with the similar quantity calculated by de Jong *et al.*[10] for Shapley–Ames galaxies and by Soifer *et al.*[5] for IR galaxies from the IRAS mini-survey. For this, we need to make three corrections: one for galactic extinction, one for internal absorption, and one for conversion from *R* to *B* magnitudes. It is probably the second of these which introduces the greatest uncertainty into the results. The first correction has already been mentioned. For the third correction we adopt $B-R=1.3$ mag, a value appropriate to face-on spirals in the catalogue of Bothun *et al.*[11]. (For 0425−012 we additionally assumed an $R-I$ value of 0.9 mag.)

The correction for internal absorption (A) is more problematic. Given the limited and exotic nature of our data, application

Table 1 Coordinates, colours and fluxes of candidate IRAS galaxies and field galaxies

Name	RA (1950)	Dec (1950)	m_I	$(R-I)$	Aperture diameter (s)	Estimated $m_{B_{0.5}}$	F_{80} (Jy)	$\frac{(\nu F_\nu)_{80}}{(\nu F_\nu)_B}$
I0404+101G1	04 h 04 min 44.85 s	10° 11′ 57.2″	18.20	0.96	14.4	19.58	2.38	209
I0413+122G2	04 13 47.30	12 17 38.2	18.27	0.90	13.2	19.59	2.80	251
I0422+009G1	04 22 55.87	00 56 18.2	16.69	0.80	16.8	18.27	1.84	49
I0425−012G1	04 25 11.36	−01 14 39.1	16.47	—	16.8	18.23	1.38	35
Other galaxies								
I0404+101G2	04 04 46.06	10 11 37.7	19.47	0.82	9.6			
I0404+101G3	04 04 40.85	10 11 30.8	17.10	1.22	16.8			
I0412+085G1	04 12 33.56	08 31 22.5	19.84	—	12.0			
I0412+085G2	04 12 33.56	08 31 35.2	19.14	—	13.6			
I0412+085G3	04 12 32.93	08 31 44.9	17.74	—	13.6			
I0413+122G1	04 13 47.59	12 17 47.4	18.30	0.98	10.8			
I0413+122G3	04 13 47.22	12 17 33.0	18.60	0.91	9.6			
I0422+009G2	04 22 53.42	00 56 39.3	17.95	0.96	16.8			
I0425−012G2	04 25 12.65	−01 13 44.5	17.33	—	12.0			
I0425−012G3	04 25 14.72	−01 14 53.2	17.94	—	13.2			
I0425−012G4	04 25 14.13	−01 15 25.5	16.89	—	12.0			

of any of the standard absorption prescriptions seems difficult. We have instead chosen to adopt $A_R = 0.3$ mag (or $A_B = 0.5$ mag). This value is typical for the 'average' inclined spiral, if we attribute the difference in the mean spiral $B-R$ colour of 1.5 mag from the face-on spiral colour of 1.3 mag in the catalogue of Bothun *et al.*[11] as arising from internal reddening. The resulting implied colour excess $E(R-I) \sim 0.1$ mag is not inconsistent with our measured colours, but we stress that because the galaxies studied here may be quite optically thick in dust, any adopted correction must be viewed with caution.

Our estimated corrected B magnitudes for the IRAS source identifications are given in Table 1, together with the (interpolated) 80-μm fluxes from Houck *et al.*[1], and the resulting $L(80\ \mu m)/L(B)$ ratios. The last cover a range of extraordinarily large values, from 35 to 251. We consider the value of 251 for 0413+122 an upper limit, though, because both the magnitude listed for the object is a probable underestimate of the total magnitude, and also because the other two galaxies within the IRAS beam may contribute significantly to the IR flux. Even if the flux is equally shared, though, a still impressive lower limit of $L(80\ \mu m)/L(B) \sim 80$ would hold for the triplet system. Figure 2 shows the IR-to-blue luminosity ratio plotted against the ratio of flux density at 100–60 μm for the galaxies studied here and for those from Soifer *et al.*[5] and de Jong *et al.*[10]. As can be seen, only one object from these other surveys falls within the high range of IR-to-blue luminosity ratio encompassed by the present sample. [Comparison with and between the de Jong *et al.*[10] and Soifer *et al.*[5] results is a little confused by the differing precepts these authors adopt for galactic reddening and internal absorption corrections. For example, applying the procedures of Soifer *et al.*[5] to M31 yields for this spiral $\log[L(80\ \mu m)/L(B)] = -1.09$, a value more in line with other low IR emitting galaxies (see Fig. 2).]

Spectroscopy

Recently, we have obtained spectra for two of the identified sources, 0422+009 and 0425−012, using the Multiple Mirror Telescope (MMT) and the 'big blue' photon counting Reticon system. Our flat-fielded, sky-subtracted, and flux-calibrated results are presented in Fig. 3. Both objects were observed on two nights (29–30 and 30–31 January 1984) for a combined integration time of 40 min for 0422+009 and 30 min for 0425−012. The 1×3 arc s slit used yielded an instrumental resolution of 5 Å, although the spectra plotted in Fig. 3 have been smoothed to ~20 Å resolution.

Two emission lines are visible in the spectrum of 0422+009, which we identify with [O II] λ3,727 and Hα. These yield a redshift $z = 0.1544 \pm 0.0005$. For a Hubble constant $H_0 = 80\ km\ s^{-1}\ Mpc^{-1}$, we obtain an estimated absolute blue magnitude of $M_{B_0,i} \simeq -20.5$, a value sufficiently bright to suggest that the large IR-to-blue luminosity ratio primarily reflects excess IR emission, and not simply great dimming of starlight by dust. At our original instrumental resolution, the [O II] line is unresolved. However, we do appear to resolve Hα, and obtain a formal FWHM value of $\sim 600\ km\ s^{-1}$, but the uncertainty in this result is large due to the poor signal-to-noise ratio. Perhaps surprisingly, no obvious emission features are present in the spectrum of 0425−012; nor is the signal-to-noise ratio adequate to show believable absorption features.

Our spectrum of 0422+009 has other notable attributes. First, it does not fit easily into any of the various empirical classification schemes that have been developed for extragalactic line emission regions[12,13]. In particular, the weakness and/or absence of both Hβ and [O II] λ5,007 is atypical of giant H II region spectra. Nor is there much evidence for the presence of [N II] λ6,584, one of the characteristic lines seen in a low excitation class of objects Heckman calls "liners"[12]. The large ratio of Hα to [O II] that is seen implies considerable reddening, a not unexpected result. The presence of line emission is itself not so unusual; for instance, about one-third of all nearby bright galaxies are liners, which are found among both spirals and ellipticals[12]. However, an intriguing aspect of the 0422+009 spectrum involves the large rest frame equivalent widths of [O II] λ3,727 (50 Å) and Hα (90 Å). Given the object's redshift, if the line-emitting region is confined solely to the nucleus, then even a conservative correction for background continuum flux would yield equivalent widths comparable with those seen in many nearby Seyferts. Clearly, long-slit spectra of high signal-to-noise ratio would help to clarify the nature of the excitation mechanism involved.

Powering the IR emission

The IRAS sources identified here seem to be extreme examples of the IR galaxies discussed by Soifer *et al.*[5]. We now briefly consider what underlying physical process powers the enormous IR output of these systems.

One possibility involves star formation bursts enshrouded by optically thick dust. Indeed, the seemingly high fraction of interacting galaxies found both here and by Soifer *et al.*[5] may (at least in some cases) provide the triggering mechanism for the outbursts. Further evidence for a thermal rather than non-thermal origin for the IR emission comes from recent 1.5 GHz Very Large Array (VLA) observations by R. Elston (personal communication), who observed but could not detect (with a 0.6 mJy 3-σ upper limit) three IR galaxies from Soifer *et al.*[5] having $L(80\ \mu m)/L(B) \sim 20$.

Rieke *et al.*[14] have presented a detailed starburst model for M82, and scaling their results up to match the properties of the IRAS source 0422+009 discussed above leads to some interesting consequences. First, the absolute IR luminosity of 0422+009 is ~35 times that of M82, and to produce such a flux from star formation, roughly 10% of the stellar mass in 0422+009 must participate in the burst. In such a case, however, non-thermal supernova remnant emission ought to be detectable in the radio[15]. Furthermore, the implied star formation rate in 0422+009 would be $100\ M_\odot\ yr^{-1}$. Our measured Hα flux of $(15 \pm 5) \times 10^{-15}\ erg\ cm^{-2}\ s^{-1}$, crudely estimated by normalizing the continuum flux in Fig. 3*a* to our *R*-band CCD photometry, is not inconsistent with such a high rate though. In fact, adopting the Hα flux calibration of Kennicutt[16] yields an estimated lower limit to the star formation rate of $\sim 15\ M_\odot\ yr^{-1}$, and correcting this value upwards for a reasonable 2–3 mag of internal absorption would produce the requisite rate.

One alternative power source might be an embedded active galaxy nucleus, such as a Seyfert nucleus, as proposed by Antonucci and Olszewski[15]. Our data provide little constraint on this possibility. However, future spectroscopic and radio observations of robust IR galaxies such as those discussed here should be able to distinguish between the starburst and active nuclei models.

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Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells

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The complete 1,210-amino acid sequence of the human epidermal growth factor (EGF) receptor precursor, deduced from cDNA clones derived from placental and A431 carcinoma cells, reveals close similarity between the entire predicted v-erb-B mRNA oncogene product and the receptor transmembrane and cytoplasmic domains. A single transmembrane region of 23 amino acids separates the extracellular EGF binding and cytoplasmic domains. The receptor gene is amplified and apparently rearranged in A431 cells, generating a truncated 2.8-kilobase mRNA which encodes only the extracellular EGF binding domain.

EPIDERMAL growth factor (EGF), a potent mitogenic polypeptide, initiates cellular responses by binding to specific receptors on the surface of target cells (for reviews see refs 1, 2). Like several other growth factors, the binding of EGF to its cell surface receptor triggers a cascade of intracellular events, including induction of a tyrosine kinase activity intrinsic to the EGF receptor[1-5]. Shortly after binding, the EGF–receptor complexes are localized in clathrin-coated regions of the plasma membrane and are then internalized by the cell.[6,7].

How these events lead to the stimulation of DNA synthesis and cellular proliferation are unknown. The recent observation that the receptors for platelet-derived growth factor (PDGF)[8,9], insulin[10,11] and insulin-like growth factor (IGF-I)[12], are also tyrosine-specific protein kinases suggested that tyrosine phosphorylation might be an important early step in triggering proliferation. The tyrosine-specific protein kinase activity of several retroviral transforming proteins (reviewed in ref. 13) has led many workers to search for a connection between growth factor receptors and transforming proteins. In their analysis of partial protein sequences, Downward *et al.*[14] demonstrated close similarity between the EGF receptor and the avian erythroblastosis virus (AEV) v-*erb-B* transforming protein[15]; evidence was presented suggesting that the viral transforming protein contained only the transmembrane and tyrosine kinase domains of the avian EGF receptor, and lacked most of the extracellular domain responsible for EGF binding. It was proposed that transformation of cells by AEV was the result, at least in part, of expression of a truncated EGF receptor molecule lacking the extracellular control domain[14].

We have now investigated EGF receptor expression in placental and A431 carcinoma cells[16]. A431 cells were chosen because they show two unusual features. First, they possess about 10–50 times more EGF receptors on their surface than most other cell types[16,17]. These high EGF receptor levels seem to be associated with the presence of a chromosomal translocation (M4) involving chromosome seven[18]. Second, their mitogenic response to EGF does not correlate with their increased EGF binding capacity. Thus while low concentrations of EGF stimulate their proliferation, EGF concentrations mitogenic for many cell lines inhibit proliferation of A431 cells[19,20]. It was therefore of great interest to investigate the genetic basis for the unusual features of this tumour cell line.

The complete amino acid sequence derived from human EGF receptor cDNAs from placental and A431 cells confirms the findings of Downward *et al.*[14], and matches partial amino acid sequences obtained from purified placental and A431 cell EGF receptor protein. Our findings also demonstrate that the EGF receptor gene is amplified in A431 cells. Furthermore, we describe the detection of an aberrant A431-specific chimaeric mRNA molecule which consists of a fragment of EGF receptor coding sequences recombined with sequences of unknown origin and function. This aberrant mRNA encodes only the external EGF binding domain of the receptor and lacks sequences which would encode the transmembrane and cytoplasmic domains. Expression of a truncated domain similar to the erb-B polypeptide found in AEV transformed cells[14] was not detected but its existence at low concentrations cannot be excluded.

Partial amino acid sequence

Receptor purified by immunoaffinity chromatography followed by gel permeation HPLC in guanidine after reduction and alkylation to cleave disulphide bonds was digested with trypsin and the peptides fractionated by preparative reverse-phase HPLC[14]. The purification and partial sequence analysis data of these peptides are shown in Fig. 1. The amino-terminal sequence of the intact receptor was deduced for a short stretch of 8 residues (see Fig. 1*v*). These partial amino acid sequences were found to be identical (apart from 2 residues) to those subsequently established by nucleotide sequencing of cDNA.

EGF receptor cDNA clones

Oligo(dT)-primed cDNA libraries were prepared from mRNAs derived from human placental tissue and epidermoid carcinoma (A431) cells using the λgt10 vector system[21]. As analysis of EGF receptor biosynthesis in A431 cells had suggested a polypeptide

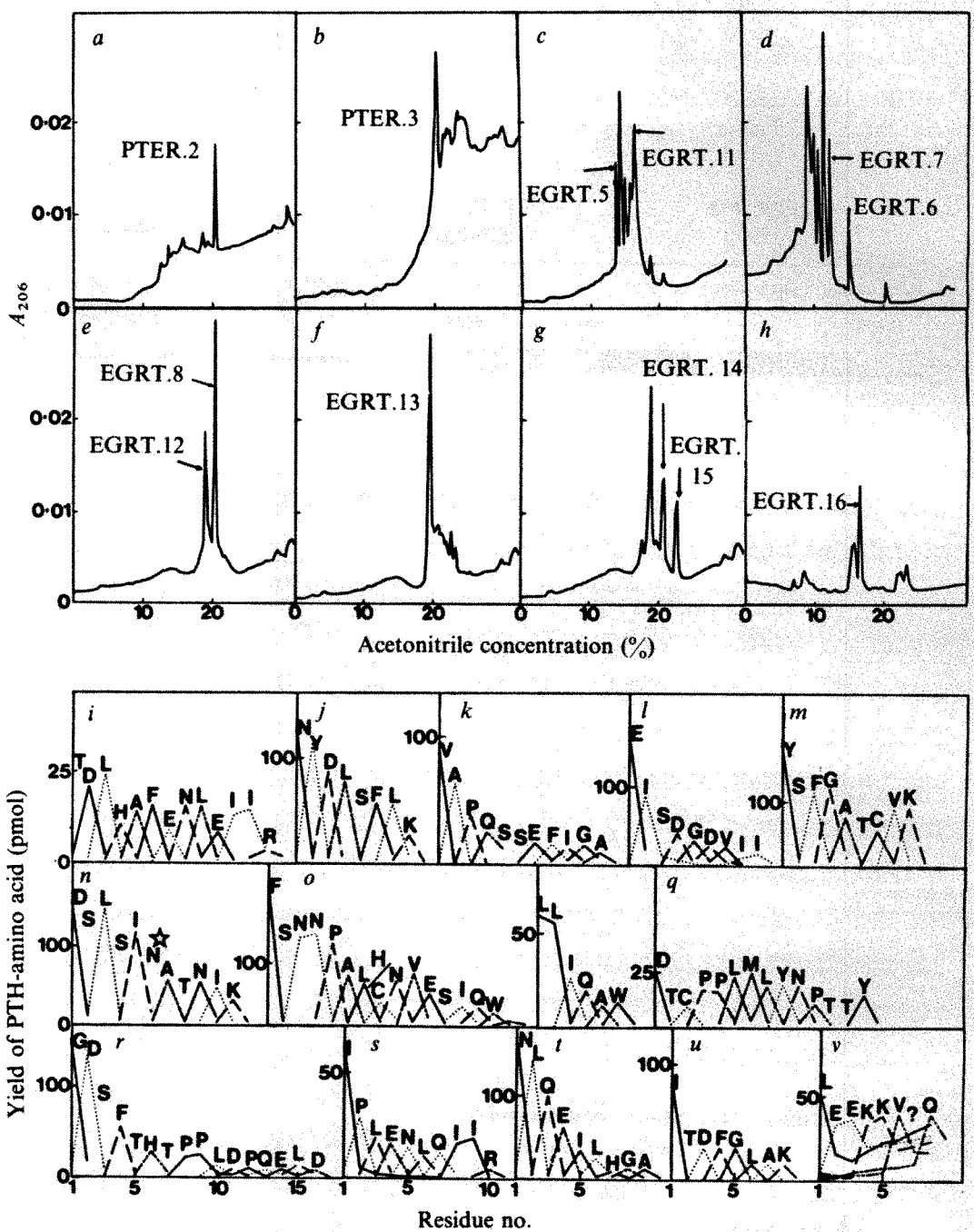


Fig. 1 EGF receptor peptide sequence analysis. *a–h*, Purification of peptides from the EGF receptor for sequence analysis. EGF receptor was purified from human placenta (*a*, *b*) or A431 human epidermoid carcinoma cells (*c–h*) using monoclonal antibody R1 (ref. 42); it was then fully reduced and alkylated, run on a gel permeation column, digested with trypsin and the resulting peptides fractionated by reverse-phase HPLC at *p*H 2.0, as described previously[14]. Fractions corresponding to optical density peaks in the first reverse-phase separation were further purified by reverse-phase HPLC on a Synchropak RPP C_{18} column (4.6 × 75 mm; Synchrom, Linden, Indiana) equilibrated in 10 mM ammonium acetate buffer, *p*H 6.5. A linear gradient of 0–45% acetonitrile over 45 min was used to elute peptides, flow rate 1 ml min^{-1}. The absorbance of the eluate was monitored at 206 nm and 0.5 ml fractions were collected. The figure shows the resulting chromatograms when the following fractions from the *p*H 2.0 fractionation were run: *a*, tryptic peptides from the placental EGF receptor eluting at 19% acetonitrile; *b*, placental peptides eluting at 22% acetonitrile; *c*, tryptic peptides from the A431 cell EGF receptor eluting at 21% acetonitrile; *d–h*, A431 peptides eluting at 18, 24, 26, 28 and 22% acetonitrile, respectively. *i–v*, Sequence analysis of peptides from the EGF receptor. Sequence determination was carried out as described[14]. The figure shows the yield of PTH derivatives of amino acids at each cycle of the Edman degradation for the peptides: *i*, PTER.2; *j*, PTER.3; *k*, EGRT.3 (for which the full purification has been described elsewhere[14]); *l*, EGRT.5; *m*, EGRT.6; *n*, EGRT.7; *o*, EGRT.8; *p*, EGRT.11; *q*, EGRT.12; *r*, EGRT.13; *s*, EGRT.14; *t*, EGRT.15; *u*, EGRT.16. *v*, The amino-terminal sequence for the whole EGF receptor molecule from A431 cells. The protein was treated with 0.05% SDS before sequencing. The seventh residue could not be assigned. ☆, The sixth residue of EGRT.7 was assigned from amino acid analysis of the peptide; the absence of asparagine-PTH at this sequencer cycle suggests that the residue is glycosylated.

backbone of molecular weight (MW) ~ 138,000 (ref. 22), at least 3,500 base pairs (bp) of coding sequence would be required, thus a sizing step was introduced to enrich for cDNA molecules greater than 2,000 bp long. For screening purposes a 51 bp-long DNA hybridization probe (Fig. 2*a*) was designed and synthesized[23] on the basis of a preliminary partial amino acid sequence of an A431 EGF receptor cyanogen bromide (CNBr) peptide (EGRC.1)[14]. Previous studies have shown that the use of a single long probe for clone screening under conditions of low stringency is often superior to the more commonly used technique which involves screening with pools of short oligonucleotide probes[24–26].

Initial screening of the placental cDNA library (2×10^5 clones) yielded seven distinct positive clones as judged by *Eco*RI restriction endonuclease mapping and Southern hybridization[27] with our synthetic probe. The longest fragment (1.4 kilobases, kb) of clone λ HER-P3 was sequenced using the dideoxynucleotide chain termination procedure[28–30]. The resulting sequence confirmed the presence of a region of similarity with our probe which, despite two errors in the preliminary determination of the amino terminus of the CNBr peptide, differed at only five positions from the sequence of the cloned cDNA (Fig. 2*a*, *c*). A second sequence analysis of this peptide gave the amino-terminal sequence Gly-Asp- (see ref. 14) and reinspection of these data revealed a trace of PTH-Asp at step 1 supporting the sequence found in analysis of cDNA clones. Comparison of the

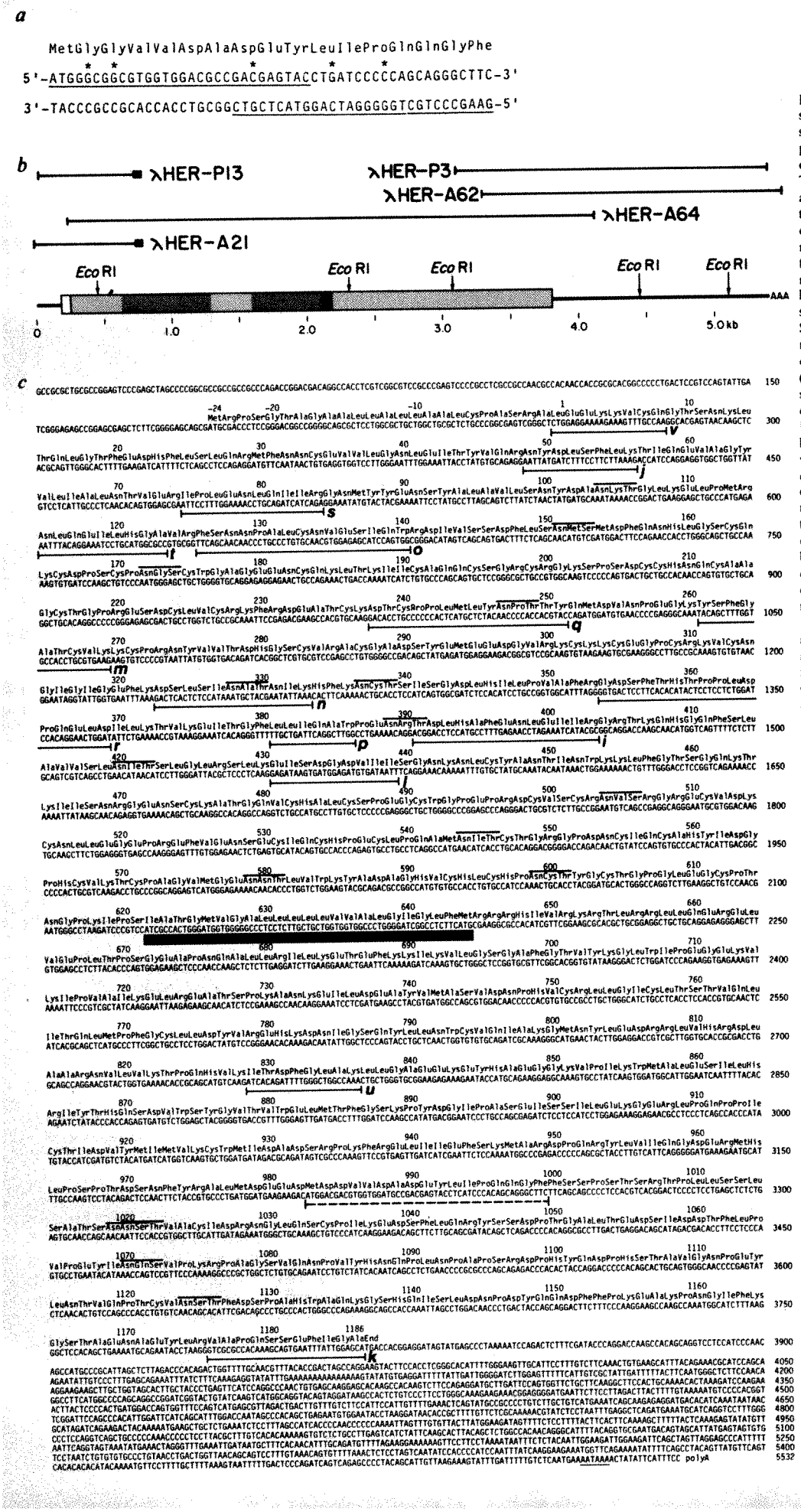


Fig. 2 EGF receptor cDNA nucleotide sequence and predicted amino acid sequence. *a*, The nucleotide sequence of probes based on the amino acid sequence of a CNBr peptide of A431 EGF receptor. The initial amino acid residue was assumed to be methionine since the peptide was generated by cyanogen bromide cleavage[14]. The DNA sequence was designed on the basis of published codon usage frequencies[57]. Two overlapping 30 residue-long oligonucleotides (underlined) from coding and non-coding strands were chemically synthesized[23]. *b*, Schematic representation of human EGF receptor cDNA clones. Overlapping clones from placenta ("P") and A431 ("A") epidermoid carcinoma cells used in sequence determination and a schematic diagram of the complete cDNA structure. Untranslated sequences are represented by a line, coding sequences are boxed. The white portion represents sequences which encode the signal peptide; hatched regions code for the mature receptor, dark hatched regions are high in cysteine residues (see text). The black region demarcates the coding region for the putative transmembrane domain. Black boxes on the 3′ ends of λ clones indicate that these clones were obtained by specific priming (complementary sequence at positions 757–780). *c*, Nucleotide and predicted amino acid sequence of EGF receptor. Nucleotides are numbered at the right; amino acids are numbered throughout. Peptide sequences derived from purified receptor preparations are underlined and labelled according to Fig. 1(*i*–*u*). The peptide underlined with a broken line was that used to design a long synthetic DNA probe (see Fig. 2*a*). Sites of potential asparagine-linked glycosylation are overlined; the putative transmembrane region is demarcated by a black bar. The AATAAA box close to the polyadenylated 3′ end of the mRNA is underlined.

Methods: *a*, The probe was radiolabelled by first phosphorylating the synthetic 30-mers using T4 polynucleotide kinase (PL Biochemicals) and [γ-^{32}P]ATP (Amersham, >5,000 Ci mmol^{-1}) followed by annealing the kinased single strands and filling in the complementary sequences using *Escherichia coli* DNA polymerase I (Klenow fragment) with [α-^{32}P]dCTP and [α-^{32}P]dATP (Amersham) yielding probes of 5×10^8 c.p.m. per μg specific activity. Hybridization was carried out in conditions published elsewhere[24]. Filters were washed at 45 °C with 0.2 × SSC, 0.1% SDS. Asterisks denote nucleotides of the synthetic probe which did not match the sequence of the isolated clone. *b*, Cytoplasmic mRNA from total human term placental tissue and A431 cells were isolated in the presence of RNase inhibitors following standard protocols[58]. cDNA synthesis[59], cloning into a λ vector (λgt10)[21], and plaque screening[60] were carried out according to published procedures. *c*, Nucleotide sequence analysis was carried out by subcloning suitable restriction fragments from our recombinant λ clones into M13-based cloning vector (M13mp10 and M13mp11[29]) followed by primed DNA synthesis on single-stranded DNA templates in the presence of dideoxynucleoside triphosphates[28,30].

```
HER      536 PECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHL
v-erb-B                        MK  F       A   L  D  R    NA  Q

HER      596 CHPNCTYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFMRRRHIVRKRTL
v-erb-B           R  K       -  S  T   A  V  G  C  V  G     YL

                                                  * * **    *
HER      656 RRLLQERELVEPLTPSGEAPNQALLRILKETEFKKIKVLGSGAFGTVYKGLWIPEGEKVK
v-erb-B                              H          V          I

            ** *     *        * **              *                      *
HER      716 IPVAIKELREATSPKANKEILDEAYVMASVDNPHVCRLLGICLTSTVQLITQLMPFGCLL
v-erb-B                                                          Y

                     *       * ** * *     **** * * **       *  ***
HER      776 DYVREHKDNIGSQYLLNWCVQIAKGMNYLEDRRLVHRDLAARNVLVKTPQHVKITDFGLA
v-erb-B       I                          E

                             * * ** * *          ***** *    *    *  **
HER      836 KLLGAEEKEYHAEGGKVPIKWMALESILHRIYTHQSDVWSYGVTVWELMTFGSKPYDGIP
v-erb-B           D

                      * * *   * *       *  **      ** *
HER      896 ASEISSILEKGERLPQPPICTIDVYMIMVKCWMIDADSRPKFRELIIEFSKMARDPQRYL
v-erb-B           V                                          A        P

HER      956 VIQGDERMHLPSPTDSNFYRALMDEEDMDDVVDADEYLIPQQGFFSSPSTSRTPLLSSLS
v-erb-B                     K  T  E   E  I       V  H    N

HER     1016 ATSNNSTVACIDRNGLQSCPIKEDSFLQRYSSDPTGALTEDSIDDTFLPVPEYINQSVPK
v-erb-B           ATN      -  GH  VR    V          NFL  E    G    A    V  LM

HER     1076 RPAG-SVQNPVYHNQPLNPAPSR----DPHYQDPHSTAVGNPEYLNTVQPTCVNSTFDSPA
v-erb-B      K  STAM   QI  -  FISLT  I  KLPM  SR   NS      D         N  SPLAKTV  E  SP

HER     1132 HWAQKGSHQISLDNPDYQQDFFPKEAKPNGIFKGSTAENAEYLRVAPQSSEFIGA
v-erb-B      Y  I  S  N    N            L  TSCS
```

Fig. 3 Comparison of EGF receptor with v-*erb-B* predicted amino acid sequences. Only non-matching amino acids are shown for v-*erb-B*. The bracket on the first line indicates the start of the v-*erb-B* sequence[15]. The putative transmembrane region is boxed; the arrow demarcates the autophosphorylated tyrosine residue in pp60^{src} (ref. 61). Asterisks indicate residues conserved between the receptor sequence and several members of the *src* gene family (*src, fes, yes, fps*). Hyphens (gaps) have been introduced for optimal alignment. The C-terminal tetrapeptide of the v-*erb-B* sequence derived from the AEV *env* gene is underlined[15].

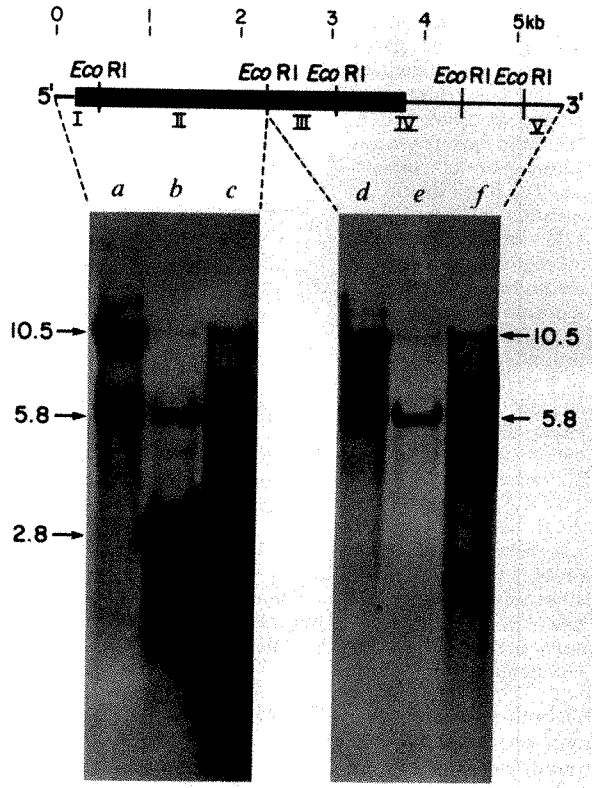


Fig. 4 Northern blot analysis of cytoplasmic placental and A431 mRNAs. Lanes *a* and *d*, placental mRNA; *b* and *e*, A431 mRNA; *c* and *f*, mRNA from a second, independently passaged line of A431 cells. Dashed lines indicate the regions of the schematically depicted cDNA giving rise to the hybridization patterns shown. Numbers on the left and right indicate sizes (in kb) of major mRNA bands. Roman numerals denote *Eco*RI fragments used as hybridization probes (see below).

Methods: A431 cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum in an atmosphere of 10% CO_2–90% air at 37 °C. Cytoplasmic mRNA isolated from frozen placental tissue after pulverization in liquid N_2 and from fresh A431 cells[58] was separated (2 μg per lane) on 1% agarose formaldehyde gels and transferred to nitrocellulose filters as described by Lehrach *et al.*[36]. Rat liver ribosomal RNA (18S = 1,874 nucleotides; 28S = 4,718 nucleotides; I. Wool, personal communication) and 42S vesicular stomatitis virus RNA (~12 kb) were used as size markers. Nitrocellulose filters were hybridized under high-stringency conditions with radiolabelled *Eco*RI fragments (I = 293 bp; II = 1,838 bp; III = 768 bp; IV = 1,373 bp; V = 432 bp) derived from clones λHER-A62 and λHER-A64.

complete sequence of the 2.3-kb cDNA clone λHER-P3 with the nucleotide sequence of the oncogene v-*erb-B*[15] showed that it coded for 243 carboxy-terminal amino acids of the receptor followed by a termination codon and about 1,600 bp of presumably 3′-untranslated sequences lacking a poly(A) homopolymer stretch (see Fig. 2*b*).

Using the 1.4 kb *Eco*RI fragment of λHER-P3 as a probe, 21 clones were isolated from an A431 cDNA library and characterized by *Eco*RI restriction analysis. Clones λHER-A62 and λHER-A64 were found to contain overlapping DNA fragments, which together spanned a stretch of 5.2 kb (Fig. 2*b*) of DNA. Complete sequence analysis revealed an open reading frame coding for 1,186 amino acid residues, including carboxy-terminal sequences identical to λHER-P3 and very similar to v-*erb-B* transforming protein. Clone λHER-A62 was found to contain the complete 3′-untranslated region (1,913 bp), as evidenced by the presence of an AATAAA sequence[31] followed by a poly(A) tail 15 nucleotides downstream.

The amino acid sequences of 13 tryptic peptides as well as those of the 6 previously reported[14] were located within the predicted amino acid sequence (see Figs 1, 2*c*). Only two differences were detected between the 230 amino acid residues determined by peptide sequencing and those derived from analysis of the cloned cDNA (Cys as opposed to His at position 133). Reinspection of amino acid sequencing data revealed a failure to distinguish between Cys and His in peptide EGRT.8 and to assign amino-terminal Asp in peptide EGRC.1. Remarkably, clone λHER-A64 starts with a nucleotide sequence encoding the amino acid sequence NH_2-Leu-Glu-Gly-Lys-Lys, which is identical to the amino-terminal sequence of the A431 receptor polypeptide (see Figs 1*b*, 2*c*).

In order to obtain missing sequences upstream from those which encode the amino-terminus of the mature protein, an A431 cDNA library generated by priming with a 24 nucleotide-long synthetic oligomer (see Fig. 2 legend) was screened with the 5′-most *Eco*RI fragment of λHER-A64. Clone λHER-A21 perfectly matched the sequence of λHER-A64 and extended it 260 bp further upstream. The new sequence contained coding information for an additional 24 predominantly hydrophobic amino acids, including the presumptive initiator methionine. This sequence probably represents the EGF receptor signal peptide which is necessary for transfer of the nascent polypeptide into the lumen of the endoplasmic reticulum. Basic and polar amino acid residues flank the hydrophobic core of the amino-terminal 24-amino acid sequence, a common structural feature of signal peptides. Furthermore, within the 186 bp of 5′-untranslated sequence, an in-frame termination codon is located 36 nucleotides upstream (Fig. 2*c*), supporting the assignment of residue −24 as the initiation methionine of the 1,210 amino acid-long EGF receptor precursor polypeptide. Placental EGF receptor clones were generated in an analogous priming experiment. Clone λHER-P13 (Fig. 2*b*) was characterized and matched perfectly the sequence of λHER-A21. These data show

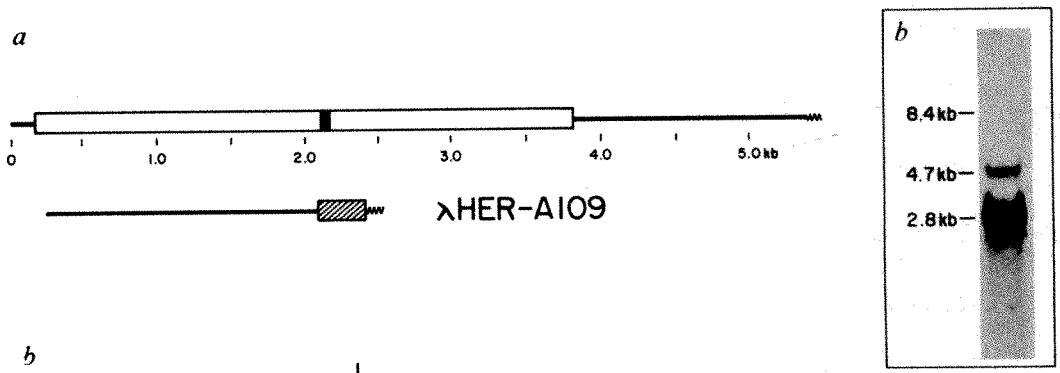


Fig. 5 Structure of an A431 cell-specific cDNA variant. *a*, Schematic map of normal (top) and variant (bottom) cDNA. The solid box represents transmembrane sequences. The black line in the variant cDNA clone λHER-A109 represents the region of identity with the normal EGF receptor sequence; the cross-hatched box represents a region of unknown origin. ~, Poly (A) tails. *b*, Northern blot analysis using a probe derived from the non-homologous region of variant cDNA clone λHER-A109. A 260-bp *Hpa*I–*Eco*RI fragment was used as radioactive probe (10^8 c.p.m. μg^{-1}). Blots were exposed for 72 h. Sizes of mRNAs are shown in kb. 28S (4,718 nucleotides) and 18S (1,874 nucleotides) ribosomal RNAs were use as size standards. No hybridization was detected in a parallel experiment with placental mRNA. *c*, Nucleotide sequence of cloned cDNA from λHER-A109. The start of the cDNA is shown, together with the junction of non-homology to EGF receptor cDNA and the entire non-homologous 3′ end.

```
b
-8                610                620                630  633
AlaLeuCys - - - - - - GluGlyCysProThrAsnGlySerTyrIleValSerHisPheProArgSerPheTyrLysMetSerValHisEnd
GCGCTCTGC - - - - - - GAAGGCTGTCCAACGAATGGAAGCTACATAGTGTCTCACTTTCCAAGATCATTCTACAAGATGTCAGTGCACTGAAACATGCAGGGGCGTGTTGAGTGTGGAAGGATC

TTGACAAGTTGTTTTGAAGATAGCATTTTGCTAAGTCCCTGAGGTCACTGGTCCTCAAAGCGGCATGGCGCATGGCGTGGCTGGTTCTGCCACATGCCAGCTGTGTGACCTCTGAGACTCCACTTCTTCC

GTGCTGAAAATAAAGAAGGAGTTTTACTAAGGACCAAACAAGATAATGAATGTGAAACTGCTCCATGAACCCCAAAGAATTATGCACATAGATGCGATCATTAAGATGCGAAGCCATCGAGTTACCACCT

GGCATGCTTAAACTGTAAAGAGTGGGTCAAAGTAAACTGAATTGGAAAATCCAAAGTTATGCAGAAAAACAATAAAGGAGATAGTAAAAAGGGTTAACGAGCCAGTCCAGGGGAAGCGAAGAAGACAAAA

AGAGTCCTTTTCTGGGCCAAGTTTGATAAATTAGGCCTCCCGACCCTTTGCTCTGTTTGCTTTATCAACTCTACTCGGCAATAACAATAAAAGACTCCAGGTGGTGTATTTGGAGTGGCTTACTG polyA
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that both placental and A431 receptors are not synthesized as larger precursor molecules aside from the signal peptide; the unmodified precursor and mature polypeptides have predicted molecular weights of 134,300 and 131,360, respectively. These values are in good agreement with the molecular weight (138,000) determined for EGF receptor synthesized in tunicamycin-treated A431 cells, where *N*-linked glycosylation does not occur[22].

Human EGF receptor/v-*erb-B* homology

Partial amino acid sequence analysis of peptides obtained from placental and A431 cell EGF receptor preparations had demonstrated that the EGF receptor matches, in part, the amino acid sequence of the product of the v-*erb-B* oncogene from avian erythroblastosis virus (AEV-H[14,15]). Availability of the complete EGF receptor sequence derived from cloned cDNA now permits a detailed sequence comparison. Figure 3 shows an alignment of the 604 residue-long v-*erb-B* sequence[15] with 631 carboxy-terminal amino acids of the human EGF receptor. Extensive amino acid sequence homology is detected over a 376 residue-long core region, beginning at the cytoplasmic junction with the transmembrane domain present in both sequences; 95% of the residues in this region are conserved, which is highly significant considering the evolutionary distance between birds and humans, and therefore probably represent sequences essential for the function of this domain. A 244 residue-long stretch within this region contains 60 residues (24.6%) which are conserved in other oncogenes of the *src* gene family (for clarity, only *src*, *fes*, *yes* and *fps* are shown in Fig. 3)[32]. These include the tyrosine which is the substrate for the autophosphorylation reaction of $pp60^{src}$ (refs 33, 34; Fig. 3). The equivalent region of the EGF receptor also retains the amino acid residues thought to be involved in the formation of an ATP binding site[32,35]. The extent of the sequence conservation is reduced in sequences flanking this 376-amino acid core region until, at residue 600, the v-*erb-B* sequence is fused to the carboxy-terminal tetrapeptide of the AEV *env* protein[15]. The EGF receptor sequence continues from the point of divergence at the 3′ end for another 32 amino acids. In the light of the sequence identity in the putative tyrosine specific kinase domain, it is surprising that the *erb-B* transforming protein has not been shown to possess kinase activity. This may reflect technical difficulties or could indicate that the carboxy-terminal region, in which there is less similarity between the sequences, may be important for expression of the EGF receptor kinase activity. The complete sequence further substantiates the hypothesis that v-*erb-B* oncogene sequences originated from cellular sequences which encode the avian EGF receptor[14]. As the nucleotide and hence the amino acid sequence of the avian EGF receptor is unknown, the relevance of particular amino acid differences between the human receptor and avian oncogene sequences to the transforming function of the virus remains unclear. The extent of similarity further supports our previous suggestion[14] that other oncogene products that have regions of amino acid sequence similar to v-*erb-B* and the EGF receptor, which form a group known as the *src* gene family, may be derived from cellular sequences encoding other receptors having tyrosine kinase activities such as the PDGF, IGF-I and insulin receptors.

Receptor-related mRNAs

To determine the size of the mRNA encoding the EGF receptor, Northern blot hybridization[36] experiments were done using cytoplasmic mRNA isolated from term placenta and from two independently propagated lines of A431 cells. The RNA preparations were probed with various segments of the cloned EGF receptor cDNA. Figure 4 shows that different patterns of hybridization were obtained with probes derived from sequences coding for the 5′-untranslated region and the extracellular domain as compared with probes derived from the cytoplasmic domain and the 3′-untranslated regions. In all experiments, two transcripts of 5.8 and 10.5 kb were detected in both A431 and placental RNA preparations, however the relative amounts of the transcripts differed between placenta and A431 cells, as well as between the two lines of A431 cells. Similarly, two transcripts of 12 and 9 kb have been detected in normal chicken embryo mRNA using v-*erb-B* probes[37]. These two transcripts could result from transcription of two closely related genes, or, alternatively, represent transcripts of different length from the same gene. Transcription of the mouse α-amylase and dihydrofolate reductase genes, for example, involves usage of multiple poly-(A) addition sites, generating mRNAs of variable lengths from a single gene[38,39]. Alternatively, variable splicing events could generate two mRNAs, perhaps with different function, from a single gene[40,41]. The variation in the relative levels of the two transcripts in placenta and the two A431 cell lines suggests that some specific mechanism may regulate their relative abundance depending on cell type, physiological condition or developmental state. Further experiments are needed to elucidate this phenomenon.

Probes derived from the 5′ half of the cDNA hybridize to a 2.8-kb mRNA present only in A431 cells. Compared with the

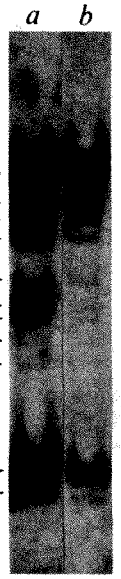


Fig. 6 Southern blot analysis of A431 (*a*) versus placental (*b*) DNA. High-molecular weight chromosomal DNA was isolated as described[58]. DNA (10 μg) was digested with excess *Eco*RI (New England Biolabs) then analysed by Southern blot hybridization. A mixture of all *Eco*RI fragments of λHER-A62 and λHER-A64 (as in Fig. 4) was radiolabelled with [α-^{32}P]dATP and [α-^{32}P]dCTP (Amersham) by the procedure of Taylor *et al.*[62]. Processed filters were exposed to X-ray film for 5 days at −70 °C using Cronex lightning plus intensifier screens. λ phage DNA was digested with *Eco*RI and *Hin*dIII was used as a size marker.

two normal mRNAs, this RNA species is overexpressed ~100-fold (Fig. 4*b*, *c*). In addition to the normal mRNA bands at 10.5 and 5.8 kb, various fainter bands are observed after long exposure times in both placenta and A431 RNA preparations (see Fig. 4 and data not shown). A 6.1-kb mRNA which is present in both A431 cells and placenta appears to be homologous to all probes used; the same is true for a placenta-specific 13-kb mRNA. Another group of mRNAs from both sources hybridizes with either 5′ (1.8 kb) or 3′ (8.9 kb) probes. Faint A431-specific hybridization signals are detected with 5′ probes at 3.3, 1.65, 1.5 and 1.3 kb in addition to the predominant 2.8-kb mRNA. These bands may represent low-abundance variant transcripts of the same gene, or possibly transcripts of other genes which are structurally related to either the entire EGF receptor or one of its major domains.

Truncated EGF receptor sequences

To characterize in detail the predominant 2.8-kb variant mRNA from A431 cells, an oligo(dT)-primed cDNA bank (>2 kb; ~10^5 clones) was screened with a 5′-specific EGF receptor cDNA probe (Fig. 4, fragment I). Of 167 positives, the three clones with the largest inserts were found by restriction enzyme analysis to be related to but distinct from clone λHER-A64. Clone λHER-A109 (2.5 kb) was sequenced and was found to be identical with our EGF receptor cDNA sequence starting at amino acid position −8 in the signal sequence and extending through almost the entire extracellular domain of the receptor. The amino acid sequence identity terminated abruptly, however, at amino acid residue 615, shortly before the transmembrane portion of the normal sequence (see Fig. 5*a*, *c*). Downstream from this residue the open reading frame continues for another 18 amino acids, followed by an opal codon (TGA) and a 548-nucleotide 3′-untranslated sequence which includes an AATAAA box 34 nucleotides upstream from the poly(A) addition site. The cDNA (λHER-A109) encodes an approximately 70,000-MW truncated EGF receptor protein which, because it contains a signal peptide but lacks the receptor transmembrane region, should be secreted by A431 cells. Monoclonal antibody R1 (ref. 42), which recognizes the extracellular domain of the EGF receptor, does in fact immunoprecipitate a secreted 115,000-MW glycopeptide, which is thought to be formed by post-translational modification of a 68,000-MW polypeptide[22]. Analogous truncation of the vesicular stomatis virus transmembrane glycoprotein by molecular cloning techniques led to secretion of a soluble protein, albeit at a severely reduced rate[43].

To establish the identity of this chimaeric cDNA, differential Northern blot hybridization experiments were carried out. Hybridization using a 185-bp *Eco*RI–*Sac*I probe derived from clone λ HER-A21, which includes only sequences upstream from the 5′ end of the variant clone λ HER-A109, resulted in a pattern identical to that seen in Fig. 4*b*, *c*. This finding, in combination with the size and sequence of clone λ HER-A109, showed that the 2.8-kb mRNA shares 5′ sequences with the 5.8- and 10.5-kb mRNAs. It is not yet clear whether transcription of these mRNAs is under the control of the same regulatory elements or whether sequence rearrangements in the 5′-flanking region of the receptor gene lead to overexpression of the 2.8-kb mRNA. A variant-specific probe (260 bp *Hpa*I–*Eco*I) derived from the 3′ end of clone λ HER-A109 hybridized to the major A431-specific 2.8-kb band as well as to fainter 4.7- and 8.4-kb bands, but not to the 5.8- and 10.5-kb mRNAs (Fig. 5*b*). Although the two less abundant (4.7- and 8.4-kb) A431-specific transcripts have the same 3′ untranslated sequence as our chimaeric cDNA clone λ HER-A109 and therefore could have engendered the cDNA, since neither of them showed homology with various EGF receptor gene sequences, we conclude that our chimaeric cDNA was derived from the 2.8-kb mRNA and that the 4.7- and 8.4-kb mRNAs probably represent transcripts from the parental gene which contain the 3′ region of the 2.8-kb mRNA.

Gene amplification

A431 epidermoid carcinoma cells express about 10–50 times more EGF receptors on their surfaces than most other cell types[16,17]. The remarkably high EGF binding capacity of these cells seems to be correlated with the presence of chromosomal translocations involving chromosome 7 (ref. 18), which is known to carry the EGF receptor gene[44,45]. In addition to the two translocated fragments of chromosome 7 (M4 and M14), A431 cells contain two copies of the normal chromosome 7 (ref. 18). To investigate further the genetic basis for the high EGF receptor levels in these cells, we compared A431 cell chromosomal DNA with DNA from placenta and other normal tissues. Assuming that the A431 cell chromosomal translocations described by Shimizu and Kondo[18] involve the entire EGF receptor gene, the signal in Southern hybridization experiments for A431 DNA was expected to be twice that detected for DNA from placenta and other human tissues. Figure 6 shows an autoradiograph of a Southern blot hybridization analysis of equal amounts of *Eco*RI-digested A431 (lane *a*) and placenta (lane *b*) DNAs. On the basis of densitometric quantification we estimate that the EGF receptor gene is amplified 15–20 times in A431 cells compared with DNA from normal diploid cells. Variable intensities of hybridization signals were observed for different bands within one lane, which may be due to different factors such as efficiency of transfer and binding to nitrocellulose, co-migration of hybridizing DNA fragments and the use of a mixed probe consisting of five DNA fragments of different size (see Fig. 6 legend). The highly selective conditions used in the hybridization experiments excluded cross-hybridization to related but non-identical sequences. Based on more detailed Southern hybridization experiments using various regions of the cDNA to probe separate blots, we estimate that the gene coding for the 5.8-kb EGF receptor mature transcript is greater than 50 kb in size (data not shown).

Our data suggest that various segments of the entire gene are amplified to the same extent. Furthermore it appears that the 8.9-kb hybridization signal obtained with A431 DNA (Fig. 6, lane *a*) is due to sequence rearrangements, since it is not present in placental and other normal DNAs (Fig. 6 and unpublished results). Additional indications of sequence rearrangements affecting the amplified receptor gene and its flanking regions have been detected in genomic Southern hybridization experiments using the 3′ nonhomologous sequence of clone λHER-A109 as a probe (A.U., in preparation).

Discussion

We have presented here for the first time the complete amino acid sequence of a cell surface receptor for a peptide ligand—the mitogen EGF.

Inspection of the receptor's predicted primary sequence reveals a stretch of 23 predominantly hydrophobic amino acids

(residues 622–644) which contains only a single amino acid with a side chain capable of hydrogen bonding. This sequence, in an α-helical configuration, could span the lipid bilayer and the group of basic residues immediately C-terminal (residues 89–101) are likely to interact with the phospholipid head groups in the membrane. It is unlikely that the external domain of the receptor contains regions that cross the lipid bilayer since in A431 cells a truncated receptor resulting from gene rearrangement and consisting of almost the entire external domain is secreted. The cytoplasmic domain may interact with the lipid bilayer but at regions with different structural features from those of the 23-amino acid transmembrane domain. The receptor therefore can be divided into two functional domains—an extracellular one of 621 amino acids and a cytoplasmic domain of 542 amino acids—linked by a short transmembrane region.

The extracellular domain would contain the EGF-binding site. This domain contains 12 of the 16 possible sites for asparagine-linked glycosylation (Asn-X-Ser or Asn-X-Thr). It is likely that the majority of these sites are indeed modified since Mayes and Waterfield[22] have shown that 11 sites are glycosylated in the precursor of the secreted external domain of the molecule.

A particularly striking feature of the receptor concerns the distribution of cysteine residues between the extracellular and cytoplasmic domains. The cytoplasmic domain of 542 amino acids contains nine cysteine residues (~2%), a value well within the range of most cytoplasmic proteins. The extracellular domain of 621 amino acids, however, contains 51 cysteine residues, most of which are concentrated within two regions of ~170 amino acids located at positions 134–313 and 446–612 (see Fig. 2*c*). Alignment of these cysteine-rich sequences with one another reveals similarities in the spacing of the individual cysteine residues, possibly reflecting the involvement of these residues in the formation of two repeated structures and a common evolutionary origin by gene duplication for this part of the receptor. We do not yet know whether these cysteine residues are all disulphide-bonded.

The cytoplasmic domain undoubtedly contains the tyrosine-specific protein kinase activity and has the stretch of amino acid sequence (approximately residues 690–940) that is shared by transforming proteins of the *src* family.

The question remains, how do the domains function in signal transduction across the membrane? If, as we suggest, the EGF receptor polypeptide crosses the membrane only once, then a signal must be transduced through this region unless two or more receptor molecules are involved or an additional polypeptide(s) mediates the response to EGF. What could be the nature of the signal? Since EGF induces rapid changes in ion movements[3] it is possible that the receptor itself could function as a channel. Very little is known about structure–function relationships for such channels except in the case of bacteriorhodopsin[46] and the acetylcholine receptor[47]. For both these proteins it seems that multiple transmembrane segments formed from either a single polypeptide in the case of bacteriorhodopsin or from multiple subunits in the case of the acetylcholine receptor contribute to channel function. Inspection of the putative EGF receptor transmembrane sequence suggests that, even if ligand-induced aggregation brought several transmembrane segments into close apposition, the sequence itself does not contain any amino acids with side chains that could be involved in ion or proton translocation.

An alternative hypothesis is that an EGF-induced conformational change in the external domain is transmitted to the cytoplasmic domain, perhaps as a result of movement of the transmembrane domain but more likely involving receptor aggregation. EGF will induce receptor clustering[48–50] and antibody cross-linking can mimic the effects of the growth factor[51,52]. Such clustering of extracellular domains would necessarily bring the corresponding cytoplasmic domains into close proximity, allowing inter-receptor interactions which might include stimulation of tyrosine kinase activity resulting in autophosphorylation. The experimental evidence cannot exclude a mechanism where a receptor molecule bearing bound ligand interacts with other polypeptides which transduce the transmembrane signal and hence stimulate kinase or other activities indirectly. However it is important to note that EGF can stimulate tyrosine kinase activity of highly purified EGF receptor (ref. 53 and Y.Y., in preparation).

Comparison of receptor gene expression patterns in normal placental tissue and the tumour cell line A431 has revealed distinct differences both at the transcriptional and at the gene structural level. We have shown that the EGF receptor is encoded by a 5.8-kb mRNA in both placental and A431 cells. We also detect a 10.5-kb mRNA which may be a transcriptional variant derived from the same gene, perhaps carrying a longer 3′-untranslated region, analogous to other genes[38,39]. Both the 5.8- and 10.5-kb mRNAs are expressed at variable but similar levels in both cells, which is remarkable because A431 cells are known to possess 10–50-fold more cell surface EGF-binding sites and associated tyrosine kinase activity than most other cell types[16,17]. The abnormality we detect instead is that A431 cells synthesize a 2.8-kb mRNA at levels 100-fold greater than that of the larger mRNA species. This mRNA encodes a 70,000-MW secreted polypeptide representing almost the entire extracellular EGF binding domain of the receptor[22]. Since this truncated receptor is secreted[22], it cannot account for the observed overexpression of EGF receptors detected by increased EGF binding capacity[1], monoclonal antibody binding[42], immunoprecipitation of tyrosine kinase activity[4,5] and receptor protein quantification as observed on SDS-polyacrylamide gel analysis of these cells[14].

Thus the important question of the mechanism for the dramatically increased EGF receptor levels in A431 cells remains unsolved. We have found that the EGF receptor gene is amplified 15–20-fold in A431 cells, yet amplification does not lead to concomitant increased synthesis of the 5.8- and 10.5-kb mRNAs. A431 cells contain two normal copies of chromosome seven and two chromosomes bearing a chromosome seven translocation[18]. Others have observed that gene amplification events are often associated with chromosomal translocations[54,55], and Shimizu and Kondo have shown that the overproduction of EGF receptor in A431 cells is genetically linked to the chromosome seven translocation (M4)[18]. We propose that the 5.8-kb and 10.5-kb mRNAs are derived from the two normal copies of chromosome seven, while the novel overexpressed 2.8-kb mRNA is derived from the amplified gene copies. Our results do not explain the link between high levels of intact receptors with chromosome 7 translocation.

Amplification of the EGF receptor gene in A431 cells could have occurred intrachromosomally or by generation of acentric extrachromosomal elements (so-called double minute chromosomes). It is possible that the sequence rearrangements we detected (A.U., in preparation) occurred during or after the translocation process and created an altered copy of the EGF receptor gene, leading to truncation and moderate overexpression, before amplification. Alternatively, sequence alterations could have occurred during gene amplification, resulting in overexpression of a single aberrant gene. Detailed analysis of the EGF receptor gene and flanking sequences from normal and A431 cells will be necessary to determine whether the occurrence of the amplified gene generates a truncated mRNA and why this mRNA is overexpressed.

It is also not clear from these results why A431 cells are uniquely unresponsive to the amounts of growth factor which are usually mitogenic for fibroblasts[19,20,56].

The finding that A431 vulval carcinoma cells produce a truncated polypeptide which is virtually identical to the external domain of the receptor is particularly intriguing in view of our earlier observation that the v-*erb-B* oncogene encodes what seems to be a truncated avian receptor polypeptide corresponding to the transmembrane and cytoplasmic domains[14]. We cannot yet exclude the presence, at a low level, in A431 cells of an mRNA which could encode such a cytoplasmic membrane-associated domain. It seems likely that the A431 2.8-kb mRNA and the secreted EGF receptor external domain are unique to

A431 cells and do not provide any clues to the neoplastic origin or character of this cell line. Since the A431 cell line—and the many variants of this cell line which must now exist (see Fig. 5)—has a multitude of defects exemplified by its 78 chromosomes[18], it is clearly best to regard this cell line with caution when exploring the mechanism of action of EGF or in characterizing changes which relate in a meaningful way to neoplasia.

The structural studies described here provide a basis from which to probe other cell lines and primary tumour tissue for alterations in the EGF receptor, its gene, or its transcription pattern.

After this manuscript was completed two publications related to the subject appeared. It was shown that A431 cells produce a 105,000-MW protein related to the external domain of the molecule[63], as previously reported by Mayes and Waterfield[22], and that amplification and altered expression of the EGF receptor gene occurs in A431 cells[64].

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Conservation and change in the DNA sequences coding for alcohol dehydrogenase in sibling species of *Drosophila*

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The DNA sequences of the alcohol dehydrogenase (Adh) *genes of four very closely related species of* Drosophila *show that the rates of nucleotide change vary greatly in different functional domains of this gene. A phylogeny of these species based on the* Adh *sequence data is consistent with that based on polytene chromosome banding patterns.*

SPECIES of *Drosophila* are ideal for studies of DNA sequence evolution as we have very detailed knowledge of the phylogenetic relationships within many groups of the genus[1]. *Drosophila melanogaster* is an unusual member of the family Drosophilidae—it is one of a very small number of species (8 out of over 2,700) that is virtually cosmopolitan in its geographical distribution. Its closest relative, *Drosophila simulans*, is also cosmopolitan, although until recently it was rare in South-East Asia and Japan[2,3]. Recent studies of the drosophilid fauna of Africa have identified six species closely related to *D. melanogaster* and *D. simulans*[4–9]. These are *Drosophila yakuba* and *Drosophila teissieri*, widely distributed in sub-Saharan Africa; *Drosophila erecta* and *Drosophila orena*, known only from tropical West Africa; and *Drosophila mauritiana* and *Drosophila sechellia*, endemic to the islands of Mauritius and the Seychelles respectively. These eight species form the *melanogaster* species subgroup. Morphologically they are very similar, their male genitalia providing their only reliable distinguishing features. Ecologically the six Afro-tropical species may be more restricted than either *D. melanogaster* or *D. simulans*: for example *D. erecta* breeds almost exclusively on the fallen

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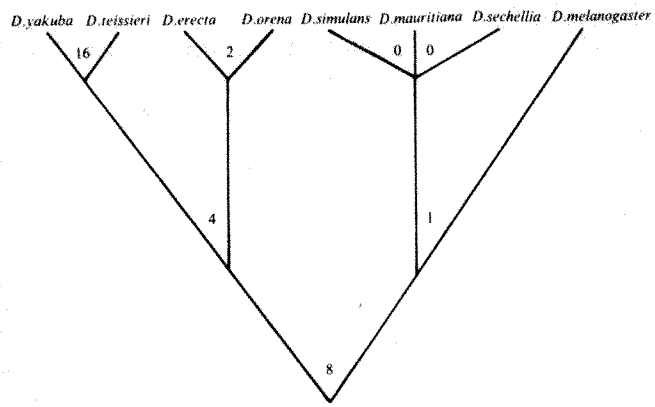


Fig. 1 The relationships among the eight species of the *D. melanogaster* species subgroup based upon their polytene chromosome banding patterns (ref. 10 and F. Lemeunier and M.A., in preparation). Within the *D. melanogaster* complex only one large inversion distinguishes the chromosomes of *melanogaster* itself from those of the other three species. The chromosomes of the members of the *yakuba* complex differ from those of the *melanogaster* complex by at least eight autosomal inversions. The numbers on the diagram indicate the minimum number of autosomal inversions that have accompanied each cladistic event.

Table 1 ADH amino acid sequence changes

	D. m (ADH-S)	*D. m* (ADH-F)	*D. sim*	*D. mau*	*D. ore*
2	Ser	Ser	Ala	Ala	Ala
28	Leu	Leu	Leu	Val	Val
51	Lys	Lys	Lys	Gln	Lys
83	Gln	Gln	Lys	Lys	Lys
85	Lys	Lys	Lys	Lys	Thr
100	His	His	His	His	Tyr
193	Lys	Thr	Lys	Lys	Lys
213	Thr	Thr	Thr	Thr	Ile
215	Pro	Pro	Pro	Pro	Ser
231	Gln	Gln	Gln	Gln	Glu
241	Gly	Gly	Gly	Ser	Gly
247	Gln	Gln	Gln	Gln	Lys
249	Thr	Thr	Thr	Thr	Ser

The predicted changes in amino acid sequence of ADH from *D. simulans* (*D. sim*), *D. mauritiana* (*D. mau*) and *D. orena* (*D. ore*) are compared with those of the ADH-S and ADH-F allozymes of *D. melanogaster* (*D. m*). The *D. melanogaster* sequences were directly determined from the proteins[21] with the exception that an extra tryptophan residue (Trp 252) was identified from the sequence of a partial cDNA clone[47]. Residues 1–33 are coded for by exon 1, 34–168 by exon 2 and 169–256 by exon 3. Residue 1 is counted as the initiating methionine. Differences from ADH-S are underlined.

fruits of the palm *Pandanus candelabrum* and *D. sechellia* on the fruits of the Rubiaceous shrub *Morinda citrifolia*[4–9].

Comparison of the polytene chromosome banding patterns of the *D. melanogaster* subgroup distinguishes two species complexes (ref. 10 and F. Lemeunier and M.A., in preparation). *D. melanogaster*, *D. simulans*, *D. mauritiana* and *D. sechellia* have similar chromosomes, only one large inversion on chromosome 3R distinguishing the chromosomes of *D. melanogaster* from those of the other species of this complex. The chromosomes of the remaining four species differ from those of the *D. melanogaster* complex by at least seven inversions. *D. yakuba*, most dissimilar from *D. melanogaster*, differs by at least 30 inversions[10]. Consideration of the inversion events required to derive the polytene chromosome banding patterns among these species gives the tree shown in Fig. 1. This hypothesis is supported by studies of hybridization within the species subgroup[11], by studies of the electrophoretic mobilities of certain enzymes[12–14], by the nature of their mitochondrial, ribosomal and satellite DNAs[15–18] and by the reaction of the species to parasitization by Cynipid wasps[19].

The *Drosophila Adh* gene

Against this background we have sequenced the genes coding for alcohol dehydrogenase (ADH; EC 1.1.1.1) from three of these species and compared them with that of *D. melanogaster* (ref. 20 and H. Haymerle and C. Benyajati, unpublished data). ADH is an abundant protein in all species of the subgroup. In *D. melanogaster* ADH is polymorphic: two electromorphs (ADH^S and ADH^F) are ubiquitous and a third ($ADH^{CH\text{-}D}$) is found in some populations. The amino acid differences between ADH^S, ADH^F and $ADH^{CH\text{-}D}$ are known, the three proteins differing in only two positions[21,22]. Kreitman[20] has sequenced the DNA of *Adh* genes from six chromosomes carrying Adh^S and five chromosomes carrying Adh^F from *D. melanogaster*. Apart from the single base pair change responsible for the lysine to threonine substitution that distinguishes ADH^S from ADH^F, these sequences differ at sites that are 'silent' with respect to the protein.

ADH activity varies during the development of *D. melanogaster*; two peaks of activity are found, the first in mature larvae and the second after eclosion of the adults[23]. The gene's transcription pattern differs between larvae and adults, although the two mRNAs encode the same protein[24]. The transcriptional organization of *Adh* in *D. melanogaster* is summarized in Fig. 2. The 'larval' and 'adult' ADH mRNAs differ in their 5′ ends. The primary adult transcript initiates 707 base pairs (bp) upstream from the larval; the removal of a 654 base intron from the primary adult transcript gives two mRNAs that differ by 50 bases in size.

Adh sequences in sibling species

Using the clones of *D. melanogaster Adh* isolated by Goldberg[25], we isolated the *Adh* genes of *D. mauritiana*, *D. simulans* and *D. orena* (see legend to Fig. 3).

The *Adh* gene sequence from these three species is compared with that of *D. melanogaster* in Fig. 3. The general structure of *Adh* is very similar, suggesting that the overall transcriptional organization is identical in the four species. The coding regions are identical in length. Table 1 summarizes the predicted amino acid changes. The amino acid sequence of the *D. simulans* ADH differs from that of *D. melanogaster* ADH^S by two residues: the N-terminal serine residue of *D. melanogaster* is replaced by an alanine residue in *D. simulans*, and the glutamine to lysine change at residue 83 accounts for the difference in electrophoretic mobility between these enzymes[26–28]. The *D. mauritiana* enzyme is predicted to differ from that of *D. melanogaster* at 5 sites and the *D. orena* enzyme at 10. Within the coding region there are 192 'effectively silent' (synonymous) sites at which a single base substitution cannot result in a coding change[20]. There are nearly 10 times as many synonymous as coding substitutions (Table 2). Overall, 20.8% of the synonymous, but only 2.2% of the non-synonymous, sites differ.

There are two coding region introns in the *Adh* gene of *D. melanogaster*, of 65 and 70 bp. The genes of the three other species have introns at identical places and of similar lengths, though in the case of *D. orena*, of quite different sequence.

Kreitman[20], in his study of 11 alleles of *D. melanogaster Adh*, found 38 polymorphisms, of which only two affected sequence length. Both of these were within the 5′ intron of the adult transcript (intron 1) and both were substitutions in Adh^F alleles that involved a partial duplication of surrounding sequences. Among our sequences, 25 of the 172 sequence variations alter length. Two of the sites of insertion within intron 1 (the 7 bp duplication at position 505 of *D. mauritiana* and the 5 bp insertion at 613 of *D. orena*) are very close to the sites of insertion in Adh^F alleles[20]: the *D. mauritiana* duplication is 1 bp 5′ to the Adh^F distal insertion and the *D. orena* insertion is 3 bp 5′ to the Adh^F proximal insertion. The longest deletion is seen in *D. orena*—this species lacks 12 bp immediately distal to the

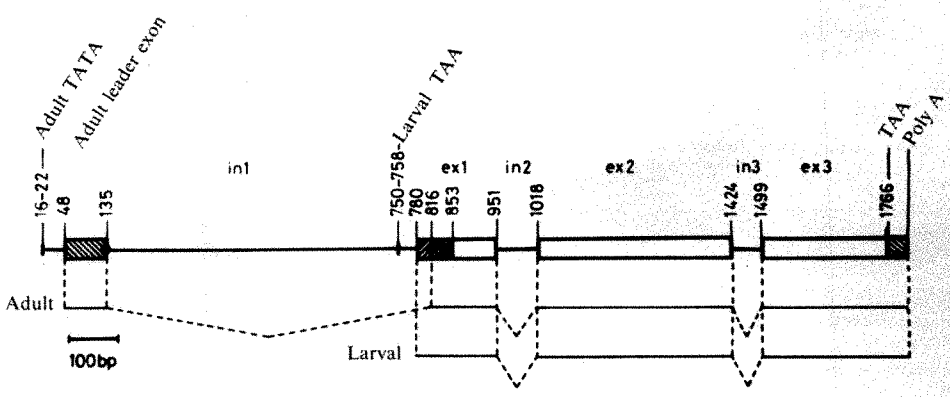


Fig. 2 The general features of the organization of the *Adh* gene of *D. melanogaster* (from refs 24 and 25). in1, in2 and in3 are the three introns and ex1, ex2 and ex3 the three coding exons. The positions of the nucleotides correspond to those of the consensus sequence shown in Fig. 3.

initiating codon. This deletion may account for the lowered levels of both ADH activity and protein in *D. orena*, which are about 30% of those of *D. melanogaster*[29].

Selective or neutral changes?

The most obvious conclusion from the data is that the rate of nucleotide substitution is higher in noncoding than in coding regions, implying selective constraints against coding region changes. Of the 768 nucleotides in the coding region, 576 (75%) could mutate to give an amino acid substitution. Between *D. melanogaster* and *D. orena*, 14.6% of the effectively silent sites have changed. We can estimate, therefore, that 84 (14.6% of 576) mutations, affecting approximately one-third of the codons, would have occurred in the coding region. Only 12 (2.2% of codons) of these have survived. This supports Kreitman's[20] conclusion that natural selection operates against coding mutations, most of which are likely to be deleterious. On the other hand the effectively silent sites show a far higher level of substitution. There are 192 sites within the coding region at which no nucleotide substitution results in an amino acid change. Forty (20.8%) of these are variable, twice the frequency of change seen in the mRNA 5′ noncoding regions.

There is significant heterogeneity in the distribution of nucleotide changes amongst the three sections of the coding region that are split by introns 2 and 3. A 3×2 contingency χ^2 test gives $\chi^2_2=9.64$; $P<0.01$. The coding region in exon 2 is the most highly conserved and that in exon 3 is the least conserved. Six of the amino acid replacements (out of a total of 12) are in the C-terminal 50 residues of the protein. It has been suggested that the coenzyme (NAD) binding sites of *D. melanogaster* ADH lies within the N-terminal 90 residues of the protein[28] and we can speculate that both this site, and the substrate binding site, lie within exon 2.

The rate of change in untranslated leader sequences is similar to that of coding DNA, and about half that seen in the intron sequences (Table 2). A similar result has been found in other studies[30,31]. This suggests that selective constraints are exerted on noncoding regions of mRNAs. They may be due to functional requirements for transcription and modification of the mRNAs, or to the demands for the interaction of other molecules with mRNA. These demands may constrain either the precise sequences of these untranslated regions or simply their secondary structure.

There is clearly a hierarchy of constraint on change in different parts of the *Adh* sequence, the percentage changes descending in the order: exon silent sites (20.8%), introns (14.1%), untranslated leader sequences (8.4%) and amino acid replacement sites (2.2%). Intron boundaries are also very highly conserved, but we cannot quantitate this as we have no rules for defining the precise limits of those sequences required for correct splicing of the primary transcript.

It is remarkable that silent sites within exons are so much more variable than the intron sequences. This unexpected complexity in the patterns of variation within the gene will be difficult to unravel until we have a better understanding of the function of intron sequences.

Evolution and phylogeny

There have been several studies of the genetic distance between members of the *D. melanogaster* species subgroup based upon the observed distribution of electrophoretic polymorphisms[12–14]. In the Hawaiian picture-winged drosophilids Carson[32] has correlated genetic distance with time, using the known age of the Hawaiian Islands. On this basis *D. melanogaster* and *D. simulans* diverged about 0.8 Myr ago, *D. simulans* and *D. mauritiana* 0.4 Myr ago and *D. melanogaster* and *D. orena* perhaps 2.0 Myr ago (assuming that the genetic distance between *D. erecta* and *D. orena* is small). However studies of electrophoretic variation within *D. melanogaster* and *D. simulans*[33–36] have shown as great a genetic distance between geographically distant populations of *D. melanogaster* as between these species. It is interesting that comparisons of the DNA sequence of *Adh* also shows a similar phenomenon, the amount of variation within *D. melanogaster* is, overall, of the same order of magnitude[20].

Table 2 Summary of *Adh* gene sequence changes

	Length (bp)	No. of changes					
		D. m	*D. sim*	*D. mau*	*D. ore*	Total	%
Adult leader	134	3	1	0	9	13	9.7
Larval leader	70	1	0	0	3	4	5.7
Subtotal	204	4	1	0	12	17	8.4
Intron 1	648	16	8	11	44	79	12.2
Intron 2	67	2	2	1	6	11	16.4
Intron 3	70	4	2	3	12	21	30.0
Subtotal	785	22	12	15	62	111	14.1
Total non-coding	989	26	13	15	74	128	12.9
Exon 1 (coding region)	96						
Synonymous	24	0	2	2	6	10	41.7
Replacement	72	1	0	1	1	3	4.2
Exon 2	405						
Synonymous	101	1	1	3	10	15	14.8
Replacement	304	1	1	1	2	4	1.3
Exon 3 (coding region)	267						
Synonymous	67	4	0	2	9	15	22.4
Replacement	200	0	0	1	5	6	3.0
Subtotal							
Synonymous	192	5	3	7	25	40	20.8
Replacement	576	2	0	3	8	13	2.2
Total coding	768	7	3	10	33	53	6.9
Grand total	1757	33	16	25	107	181	
%		1.9	0.9	1.4	6.1	10.3	

The numbers of changes from the consensus sequence are shown for the *Adh* genes of *D. melanogaster* (*D. m*), *D. simulans* (*D. sim*), *D. mauritiana* (*D. mau*) and *D. orena* (*D. ore*). Additions and deletions of more than one contiguous base are counted as one change.

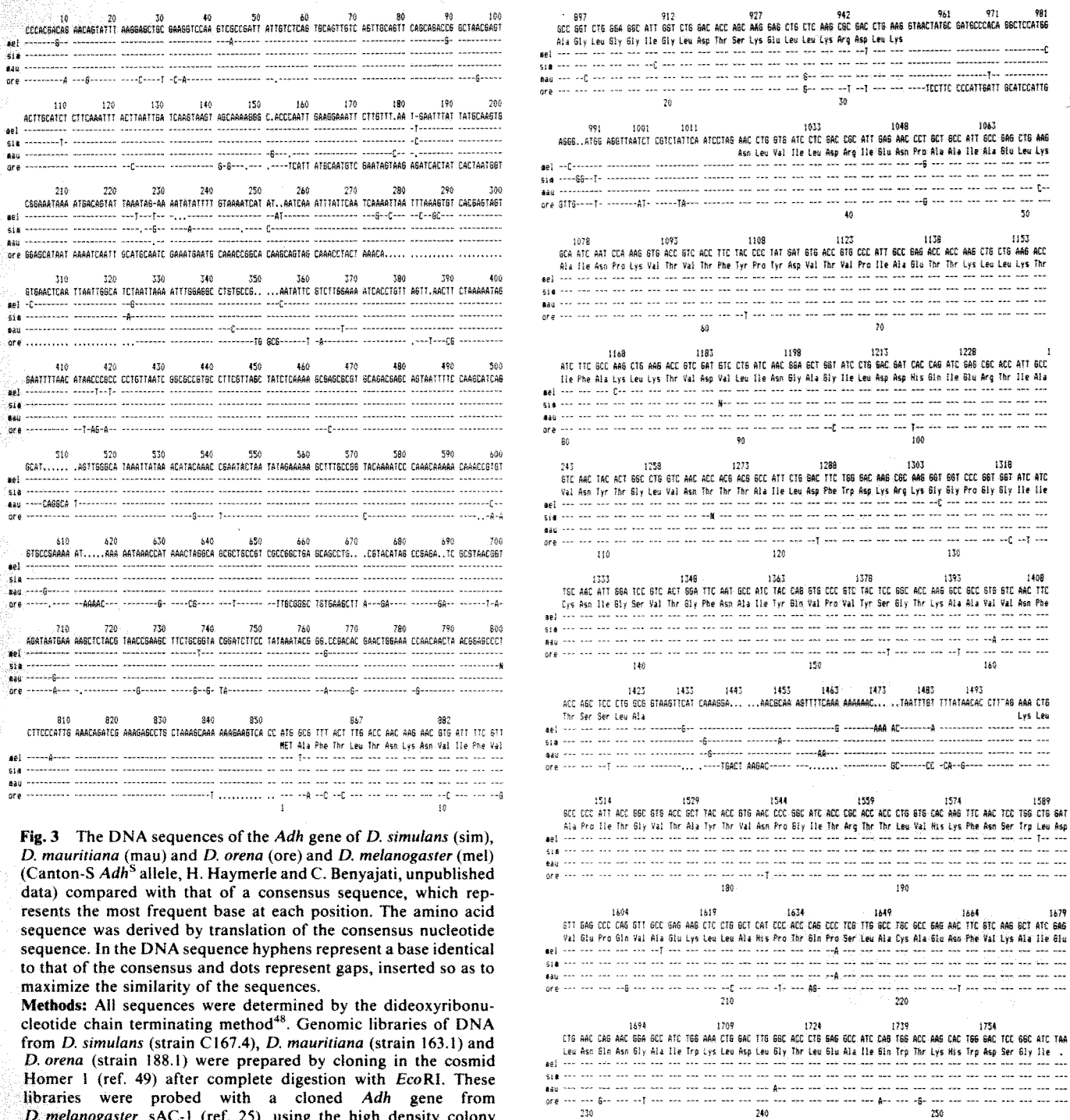


Fig. 3 The DNA sequences of the *Adh* gene of *D. simulans* (sim), *D. mauritiana* (mau) and *D. orena* (ore) and *D. melanogaster* (mel) (Canton-S Adh^S allele, H. Haymerle and C. Benyajati, unpublished data) compared with that of a consensus sequence, which represents the most frequent base at each position. The amino acid sequence was derived by translation of the consensus nucleotide sequence. In the DNA sequence hyphens represent a base identical to that of the consensus and dots represent gaps, inserted so as to maximize the similarity of the sequences.

Methods: All sequences were determined by the dideoxyribonucleotide chain terminating method[48]. Genomic libraries of DNA from *D. simulans* (strain C167.4), *D. mauritiana* (strain 163.1) and *D. orena* (strain 188.1) were prepared by cloning in the cosmid Homer 1 (ref. 49) after complete digestion with *Eco*RI. These libraries were probed with a cloned *Adh* gene from *D. melanogaster*, sAC-1 (ref. 25), using the high density colony hybridization procedure[50]. Hybridization of the *Adh* clones of these species to whole genomic DNA, digested with *Eco*RI, confirmed that the fragments cloned were of the same size as the single genomic fragments containing homology to sAC-1. The *D. simulans* sequence was obtained by sonicating the 4.79 kilobase (kb) *Eco*RI genomic fragment of this species that is homologous to sAC-1, and 'shotgun' cloning the fragments into M13mp8 (ref. 51). The *D. mauritiana* sequence was obtained in part by random shotgun clones of the 4.79 kb *Eco*RI fragment homologous to sAC-1 and in part from subclones in M13 made from *Hpa*II digestion of the *Eco*RI clone. We assumed, and then verified, that the *Hpa*II restriction sites of the 4.79 kb *Eco*RI clones of *D. melanogaster* and *D. mauritiana* are similarly distributed. The final 10 bp of *D. mauritiana* (from the *Hpa*II site at 1,756) was not sequenced. The size of the ADH polypeptide of this species, obtained by *in vitro* translation of its mRNA, is indistinguishable from those of the other species of this subgroup[29]. The *D. orena* sequence was derived from *Alu*I and *Hae*III restriction fragments of a 12 kb *Eco*RI clone (in pAT513) that included the entire *Adh* gene of this species. Subclones in M13 were identified by colony hybridization with a 2.37 kb *Sal*I–*Xba*I fragment of sAC-1.

as the difference between single *D. melanogaster* and *D. simulans* alleles.

Miyata[37] has shown that in mammals the rate of synonymous codon substitution is rather uniform, at $\sim 5.4 \times 10^{-9}$ per nucleotide per year. If we use this value to estimate the time of divergence of the species of the *D. melanogaster* species subgroup then we obtain times greater than those suggested by the electrophoretic data: 3.9 Myr for *D. melanogaster–D. simulans*, 2.9 Myr for *D. simulans–D. mauritiana* and 13 Myr for *D. melanogaster–D. orena*.

These estimates will be biased by the longer generation time of mammals than *Drosophila*. Moreover, we cannot assume that synonymous substitutions are neutral. Codon usage in *Adh* is nonrandom. This is most clearly seen in a deficit in codons ending in A and T, and a corresponding excess of G and C—a pattern which is also seen in other sequenced *Drosophila* genes

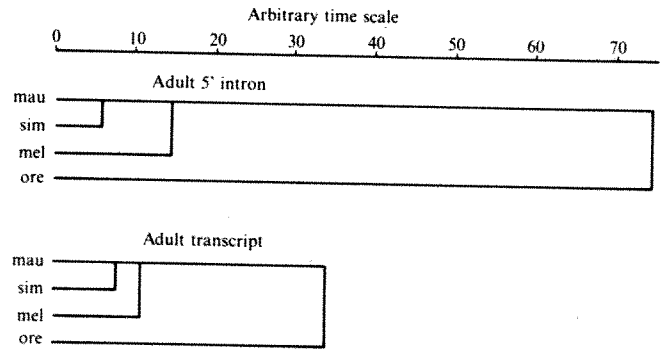


Fig. 4 Trees of the phylogenetic relationships of *D. melanogaster*, *D. simulans*, *D. mauritiana* and *D. orena* generated by M. J. Bishop's DNAPEQ computer program. The model used by this program makes two assumptions: (1) The genome is under a constant mutational threat, damage being repaired by proofreading functions of DNA polymerases. The model then assumes a constant failure rate of repair at any individual site (u, which is independent of any previous events at that site) and calculates a probability ($1-e^{-ut}$) that failure will occur over time t. This is termed the exponential failure law. (2) There is an equiprobable chance of replacement by A, C, T or G (allowing for a base to be replaced by itself). The calculation requires that the sequences be aligned for maximum homology. It regards all changes as single base substitutions, and all additions, deletions or replacements are removed. The upper tree is for sequence comparisons of intron 1, the lower tree compares the adult transcripts from their initiation sites with the termination codons. The time scale is arbitrary and could only be transformed to real time were the rates of mutation (u) known.

(M.A., unpublished data). Nonrandom codon usage is well known in other species (see, for example, ref. 38) and is especially marked in genes whose products are, like ADH in *Drosophila*, abundant[39,40]. In *Drosophila* the pattern of nonrandomness differs from that of many other eukaryotes due to there being no strong deficit of C·G doublets (M.A., unpublished data), correlating with the very low amount of the modified base 5-methylcytosine in this species[41–43]. The frequencies of transition and transversion changes among the four sequences are heavily biased towards transitions (Table 3). This is not surprising for the coding regions, as no pyrimidine, and only two purine transitions cause amino acid replacements, but it was unexpected for noncoding regions, which show a 0.75 ratio of transitions : transversions rather than the expected 0.5. In the coding region there is an excess of pyrimidine transitions: 65% of the third positions are pyrimidine, but 82% of the mutations are pyrimidine transitions.

Our data clearly confirm the relationships between the four species of the *D. melanogaster* species subgroup shown in Fig. 2. The sequence of the *Adh* gene of *D. simulans* is closer to that of Adh^S of *D. melanogaster* than to Adh^F. Kreitman's comparison of Adh^S and Adh^F alleles shows 17 sites at which a consensus Adh^S sequence differs from a consensus Adh^F sequence. *D. simulans Adh* resembles Adh^S at 14 of these sites (including Lys 193) and resembles Adh^F at the other three sites (which are not clustered). As Adh^F alleles are relatively rare in the more tropical populations of *D. melanogaster*[44,45], these facts point to Adh^F as being a more recent allele than Adh^S.

Numerical methods of constructing phylogenies have been developed which allow a probabilistic assessment of the relationships among a group of species based upon a defined subset of their attributes[46]. Recently these models have been adapted to make use of nucleotide sequence data (M.J. Bishop & A. E. Friday, personal communication) and a pairwise method of phylogeny analysis has been applied to these data from the *D. melanogaster* species subgroup. We used a program (DNAPEQ) that evaluates a maximum likelihood estimate of divergence times for pairs of DNA sequences. This program uses an exponential failure model of DNA copying error, assuming an equiprobable replacement at a site by any of the four bases. DNAPEQ calculates a matrix of pairwise divergence times (on an arbitrary time scale) and these are then used to construct a tree which best fits the pairwise comparisons. Two subsets of the sequence data for the four species were analysed: one was the adult transcript, from its initiation site to the termination codon, and the other was intron 1. The two trees generated are shown in Fig. 4. They are topologically identical, although the estimates of relative divergence times differ. The tree based on the adult transcript sequence places *D. orena* half the distance from the other species as that based on intron 1. This, of course, is entirely consistent with the different levels of variation seen in these regions of nucleotide sequence. Thus, using independently defined criteria for the construction of cladograms based upon DNA sequence comparisons, the phylogenetic relationships seen in all previous studies are fully confirmed.

Table 3 *Adh* gene transitions and transversions

	Coding	Noncoding
Transitions (t)		
C↔T	24	18
G↔A	5	15
Sum	29	33
Transversions (v)		
T↔G	4	8
T↔A	2	12
C↔A	9	11
C↔G	6	13
Sum	21	44
Ratio (t/v)	1.38	0.75

The numbers are the sums over the four species of the *D. melanogaster* subgroup of single base changes from the consensus sequence shown in Fig. 3.

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LETTERS TO NATURE

The ultraluminous interacting galaxy NGC6240

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In the course of checking preliminary lists of IRAS (Infrared Astronomical Satellite) sources to see whether any of the interacting galaxies that we have been studying[1] were included, we discovered that the position of the IRAS source 1650+024PO4 (ref. 2) is within a few arc seconds of one of our programme interacting galaxies, NGC6240. Examination of the Palomar sky survey prints shows that NGC6240 is the only non-stellar object within the 1 arc min IRAS error box for this source. Photometry at 10 and 20 μm from the United Kingdom IR Telescope (UKIRT) confirms that this galaxy is among the most luminous IR galaxies yet discovered. An interaction-induced burst of star formation is the most likely source of the strong IR emission.

Photometry of NGC6240 using UKIRT was obtained on 29 February 1984. We first found the peak in the signal from NGC6240 at K (2.2 μm), and we used this position for the longer wavelength observations. This position coincides within our measurement errors of ~2 arc s, with the optical and radio positions discussed below. Our photometric results, obtained in a 4 arc s aperture, are shown in Fig. 1, together with the published preliminary IRAS data, and available optical and radio data. This reveals that the IRAS source is indeed NGC6240.

The nuclear 10-μm luminosity of NGC 6240, inferred from our UKIRT photometry in a 4 arc s aperture, is $1.2\times10^{10}\ L_{\odot}$, based on the measured redshift[3] (7,597 km s^{-1}) and a Hubble constant H_0 of 50 km s^{-1} Mpc^{-1}. Integration of the spectrum shown in Fig. 1 yields a bolometric luminosity (L_{bol}) of $2.4\times10^{12}\ L_{\odot}$. Most of this energy is emitted in the IR, with the optical and radio emission contributing only ~10% of the total. This interacting galaxy therefore has an IR luminosity 60 times larger than the archetypal starburst galaxies M82 and NGC253 (ref. 4), for which $L_{bol}\sim4\times10^{10}\ L_{\odot}$. Its IR luminosity is an order of magnitude larger than that of the Seyfert galaxy NGC1068 (ref. 5), and comparable with that of Mkn 231 (ref. 6), the most luminous IR galaxy known.

The IR luminosity is most easily understood as arising from a massive burst of star formation, in which the radiation from young, early-type stars is thermalized by dust, and re-radiated in the IR. The quasi-thermal IR spectrum, peaking at ~100 μm, is typical of that observed in known starburst galaxies. Comparing our 10- and 20-μm fluxes in a 4 arc s aperture with the IRAS photometry at 12.5 and 25 μm in a much larger aperture, suggests that about half of the IR emission comes from a region outside the central 4 arc s. The spatial extent of this emission rules out the possibility that the dust is heated by a single compact object in the nucleus, as has been suggested for some Seyfert galaxies.

However, NGC6240 is clearly not just another starburst galaxy. It is at least an order of magnitude more luminous than typical starburst galaxies, and the recent star formation appears to be extended over an enormous region in excess of 3 kpc, significantly larger than is generally found for starburst galaxies.

A comprehensive picture of the nature of NGC6240 can be obtained using available optical and radio studies. Photographs presented by Fosbury and Wall[7] reveal that it is an extremely chaotic interacting galaxy, with a prominent dust lane and three long plumes extending from the central region. Charge-coupled device (CCD) images obtained by Fried and Schulz[8] show two distinct nuclei separated by ~2 arc s. The spectra of Fosbury and Wall, and Fried and Schulz, show intense optical emission lines arising from a region extending over at least 10 arc s. These authors have used the optical emission line intensities to distinguish between different excitation mechanisms (see ref. 9), and they find that the optical emission is characteristic of shock excitation.

NGC6240 is also a very bright radio source with a luminosity at 1,413 MHz (ref. 10) of 4.2×10^{22} W Hz^{-1} Sr^{-1}. The Very Large Array (VLA) maps of Condon *et al.*[10] show that the radio emission is coextensive with the region of intense optical line emission. The two nuclei are also clearly resolved at 4,885 MHz. Other bright radio galaxies in the Condon *et al.* sample have an IR (10 μm)-to-radio (1,445 MHz) flux density ratio of ~4. They suggest that this relation can be understood in terms of a starburst model, with the radio emission arising from supernova remnants associated with the starburst. Comparing our 10-μm flux density with the radio flux density for NGC6240 measured by Condon *et al.* for the central 5 arc s gives a ratio of 0.5. Evidently a simple starburst model is inadequate for NGC6240, and an additional energy source for the radio emission is required. Fosbury and Wall[7] suggest that the strong radio emission in NGC6240 is due to relativistic particles accelerated in the shock fronts. This interpretation appears to be more

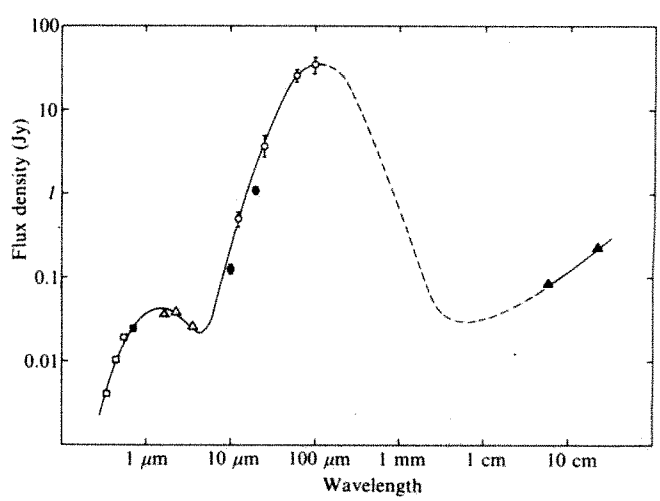


Fig. 1 Continuum spectrum of NGC6240. Data from: ●, this paper; ○, ref. 2; □, ref. 7; ■, ref. 8; △, ref. 15 and ▲, ref. 10. The dashed interpolation is a Rayleigh–Jeans Spectrum with emissivity proportional to λ^{-1} joined smoothly to the data at longer and shorter wavelengths.

consistent with the small IR-to-radio flux density ratio we find for this galaxy.

The physical picture of NGC6240 which emerges from all of these studies is that of an interaction between two gas-rich galaxies. If the impact parameter is small enough, dynamical friction will be adequate to dissipate the orbital kinetic energy and a merger of the two galaxies will ensue, as discussed by Toomre[11] and others. Such interactions produce large non-circular gas motions and infall of material as the colliding galactic nuclei spiral towards each other. The resulting shocks in this material can trigger a massive burst of star formation. NGC6240 seems to be a clear example of this process. The two nuclei, and the strong evidence of shock excitation on large scales, support this model. Furthermore, the thermal character of the IR spectrum, the very large IR luminosity, the spatial extent of the IR emission, and the corresponding spatial extent of the ionized gas are strong circumstantial evidence of a massive burst of star formation, fuelled and triggered by the interaction.

These physical processes and events in NGC6240 are similar to those which we[1] and others (see, for example, ref. 12) are finding in studies of interacting galaxies. NGC6240 is remarkable because it is such a spectacular and energetic example.

There is mounting evidence that interactions are associated with other types of active galactic nuclei[13,14]. The properties of NGC6240 outlined above demonstrate that this association has physical plausibility. In particular, interaction-induced starbursts are capable of supplying luminosities at least as large as those characteristic of all but the most luminous active galaxies.

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Solid white dwarfs as Type I supernova progenitors

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Mass-accreting white dwarfs in close binary systems are generally thought to be Type I supernova (SN I) progenitors. Low-mass ($M_{tot} \lesssim 5\,M_\odot$) binary X-ray sources (also known as Type II sources) equally appear to be the descendants of cataclysmic variables (CV) and thus to have been produced by the collapse of a mass-accreting white dwarf. We point out here that a single process, thermonuclear ignition inside a partially solid, carbon–oxygen white dwarf, may account for the full range of phenomena: from mildly or non-explosive collapses leaving a condensed remnant up to 'slow' to 'fast' SN I outbursts with total disruption of the parent star.

Type I supernovae (SN I) display a high degree of photometric and spectroscopic homogeneity[1]. The radioactive decay model[2,3] successfully accounts for their characteristic light curves. Between 0.2 and 1.0 $M_\odot$ of ^{56}Ni should be synthesized in each explosion; the exact values depend on the adopted value of the Hubble constant: $H_0 \sim 100\ \mathrm{km\ s^{-1}\ Mpc^{-1}}$ would correspond to the lowest masses and would imply a condensed remnant being left by the explosion[5]. Despite the aforementioned homogeneity, some range of variation is detected among the outbursts[6]: 'slow' supernovae show higher peak luminosities, higher velocities in their ejecta and slower declines in their light curves; 'fast' supernovae are dimmer, the velocities of the expanding material are lower and the light curve's decay is faster. Those characteristics are well fitted by Arnett's analytical models[7]. An 'effective diffusion time' τ_m is defined:

$$\tau_m \propto (M_{ej}^3 / M_{Ni})^{1/4}$$

where M_{ej} and M_{Ni} are, respectively, the masses of the ejecta and of the synthesized ^{56}Ni. Larger τ_m values mean slower light curve declines. The expansion velocities and the peak luminosities are also related to the above quantities by

$$v_{ej} \propto (M_{Ni}/M_{ej})^{1/2}$$

and

$$L_{peak} \propto M_{Ni}$$

The most fashionable model for explaining the outbursts involves central deflagration of a white dwarf in a close binary system, leaving no remnant[8], but this model implies a 'unique' configuration and does not allow for variation (M_{ej} coincides with M_{tot} and the latter is roughly the Chandrasekhar's mass). Different explosion mechanisms (helium detonation, helium–carbon detonation, and so on) may then be invoked, but their predictions contradict the observations, as almost no intermediate mass elements are ejected in those models. A natural way for fitting the light curves is, however, to assume that M_{ej} is a variable fraction of M_{tot}. Other types of observations, as positron events at the galactic centre[9,10], also suggest thermonuclear explosion with relatively small amounts of ejecta[11].

Type II galactic X-ray sources (comprising globular cluster sources, galactic bulge sources, Sco X-1 type sources, and X-ray bursters) share the common characteristic of involving a neutron star with a low-mass ($M \leqslant 1\ M_\odot$) companion. While the capture mechanism might be a valid explanation for the globular cluster sources, it cannot explain the other kind of sources. Besides, the distribution of X-ray galactic bulge sources and that of the novae in M31 show a remarkable coincidence[12]. Collapse of a white dwarf in a cataclysmic variable is the most likely formation mechanism[13].

We thus have a situation which strongly suggests that mass accreting white dwarfs in close binary systems may at some point collapse and/or explode leaving condensed remnants of various masses: from practically the same mass as the collapsing white dwarf up to zero mass in the case of complete disruption. In contrast, there is almost no model which predicts such a continuous range of behaviour.

The effects of solidification in cooling carbon–oxygen white dwarfs have been pointed out[14]. One possibility is that carbon/oxygen separation happens at phase transition: oxygen 'snow' falling to the centre and carbon being rejected to the outer layers. The likely outcomes[15] are either collapse initiated by the electron captures on oxygen at the centre or off-centre carbon ignition. With the lack of a detailed numerical simulation of the liquid/solid phase transition, we consider other alternatives, such as 'percolation' in the solid phase[16]: the free energy is lower when the carbon ions imbedded in an oxygen lattice are surrounded by oxygen ions. So, if carbon were even only slightly less abundant than oxygen at the end of the helium burning (as the recent revision of the $^{12}C(\alpha,\gamma)^{16}O$ rate suggests[17]), the relevant rate for the ignition would be that of $^{12}C + {}^{16}O$ instead of $^{12}C + {}^{12}C$. Central ignition would occur when the central density $\rho_C \sim 10^{10}\ \mathrm{g\ cm^{-3}}$ and would also lead to

collapse. But even if carbon were more abundant than oxygen and/or no separation mechanism were operating solidification would still ensure at least partial collapse. This is because when thermonuclear ignition happens in the middle of a solid, the burning propagates by conduction (the successively melted layers only afterwards become convective). The characteristic speeds are between 10^{-4} and 10^{-5} times the local speed of sound[18]. Taking into account the new corrections to the electron screening factors[19], the ignition density of the reaction $^{12}C+^{12}C$ becomes $\rho_c \sim 3.5\times10^9$ g cm^{-3}. For instance, assuming the interior to be solid from the centre out to a radius 5×10^7 cm (about half the stellar radius), it takes ~500 s for the conductive burning front to reach the edge of the solid core (in contrast with ~1 s if we had assumed a convectively-driven front, or ~0.1 s for a detonation wave). Even at those relatively low ignition densities, the electron captures on the matter in nuclear statistical equilibrium (NSE)[20] are fast enough to remove pressure efficiently and start the collapse: $\tau_e = = Y_e/\dot{Y}_e \leqslant 5$ s (where Y_e is the electron mole number). When the burning front reaches the fluid layers, it changes into a convectively-driven front and accelerates.

Calculations were performed assuming central ignition in a white dwarf with a central density of 3×10^9 g cm^{-3}, with a solid core of one-tenth of the total mass, composed of a mixture of equal parts of carbon and oxygen. While the burning propagates through the solid core at a velocity of 2 km s^{-1} hydrostatic equilibrium was assumed. When the burning front reaches the solid/liquid transition, the material between that point and the layer corresponding to 1 $M_\odot$ was instantaneously incinerated. Then the hydrodynamical behaviour was followed with an explicit numerical code[21].

Our results are preliminary because we have extrapolated the approximations used for the matter in NSE to higher densities and lower Y_e than prescribed, and the hydrostatic/hydrodynamic transition should be treated more carefully. In the same way, burning propagation in the solid, as well as in the fluid, layers needs a more detailed treatment. Keeping these restrictions in mind, we conclude that the liquid layers are ejected and the solid/fluid transition sets a lower limit to the mass of the bounded remnant. Due to the low densities of those layers ($\rho \leqslant 2.35\times10^9$ g cm^{-3}) the neutron excess of the ejected matter is not too high ($\langle\eta\rangle \sim 3\times10^{-3}$).

Thus, the mere solidification of the star's interior appears to determine the implosion of the frozen layers on carbon ignition. The burning front, when reaching the still fluid layers mimics an off-centre ignition. A single parameter (the size of the solid core, resulting from the interplay between the cooling time before mass transfer and the heating effects of the accretion process[22]) determines the amount of matter ejected. So, the whole range from mildly explosive collapse (leading to the formation of Type II X-ray sources) to 'fast' and 'slow' SN I might be covered by a single mechanism based on a well understood physical process.

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Topography of the shield volcano, Olympus Mons on Mars

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Olympus Mons, one of the largest known shield volcanoes in the Solar System, covers an area of $>3.2\times10^5$ km^2 and has a diameter of >600 km, excluding its vast aureole deposits. The structure is five times larger than the largest shield volcano on the Earth. It is situated on the north-west flank of the Tharsis volcanic region, a broad topographic rise on the martian surface. The volcano has three physical subdivisions: the summit caldera, the terraced upper flanks, and the lower flanks, which terminate in a scarp 2–10 km high that nearly surrounds the structure. A large block of images of the Tharsis region, including Olympus Mons, was obtained by the Viking mission[1]. Here we present a topographic map of Olympus Mons, compiled using various combinations of stereo pairs of these images, together with stereoscopic perspective views generated by image processing techniques.

The contour map of Olympus Mons was compiled at a scale of 1:1,000,000 with a contour interval of 200 m, using 91 stereoscopic models of Viking Orbiter photographs[2]. Special photogrammetric techniques were used for this compilation. These techniques had been developed to use the Viking Orbiter photographs, which have extremely narrow field-of-view[3], for the systematic mapping of Mars. Three hundred and sixteen control points were produced from a block of 103 photographs using analytical aerotriangulation methods. Digital elevation data were collected from stereo models on the analytical plotter at the time of map compilation. Using techniques of digital image processing[4] shown in Fig. 2, perspective stereoscopic views were made at various vertical viewing angles from different desired azimuths. The summit caldera and the terraced upper flank are viewed monoscopically from the east and the west in Fig. 2*a* and *b*, respectively; the scarps of the lower flanks are viewed stereoscopically from the north and the south-east in Fig. 2*c* and *d*.

Previous estimates of the height of Olympus Mons ranged from 17 to 23 km above the surrounding area[5–12]. A height of 27 km above the Mars topographic datum[13] is shown on the US Geological Survey 1:25 million scale, global topographic map of Mars[14], which was compiled by synthesizing remotely-sensed data from the Mariner 9 Mars mission and Earth-based radar observations[15–16]. The newly compiled topographic map (Fig. 1), shows the elevation of Olympus Mons to be 26,400 m above the Mars datum. The volume determination of Olympus Mons calculated for individual segments bounded by contour lines on Fig. 1 is given in Table 1.

Olympus Mons has the general form and slope of a classic terrestrial basaltic shield volcano[8,17,18]. Major topographic features include a complex caldera at the summit that is ~80 km across, upper flanks that form a concentric pattern of terraced terrain, and a steep basal scarp. The summit caldera is composed of at least five coalesced collapse craters[19]. Concentric terraces on the upper flanks are prominent on the east, south-east, west, and south-west sides[20]. Typically, the terraces are spaced 15–20 km apart. The unique, nearly continuous, basal scarp rises more than 10 km above the basal plains on the north and north-west, (Fig. 1); on the south-east, the scarp rises 4–5 km

Table 1 Volume determination of Olympus Mons

	Elevation (km)	Volume of segment	Accumulated ($\times 10^6$ km^3)	Elevation (km)	Volume of segment	Accumulated ($\times 10^6$ km^3)	Elevation (km)	Volume of segment	Accumulated ($\times 10^6$ km^3)
Above	26	0.0004	0.0004	17	0.073	0.336	9	0.223	1.553
	25	0.006	0.007	16	0.088	0.424	8	9.236	1.789
	24	0.013	0.020	15	0.103	0.527	7	0.247	2.036
	23	0.022	0.042	14	0.120	0.647	6	0.262	2.298
	22	0.029	0.071	13	0.140	0.787	5	0.296	2.594
	21	0.035	0.106	12	0.158	0.945	4	0.365	2.959
	20	0.043	0.149	11	0.180	1.125	3	0.436	3.395
	19	0.052	0.201	10	0.205	1.330	2	0.467	3.862
	18	0.061	0.263						

Volumes were calculated, using a planimeter, for individual segments bounded by contour lines on the map (Fig. 1). The third column lists cumulative volumes, measured downward from the top in increments of 1 km. Blasius[7,8] reported a total volume of 2.7×10^6 km^3 for Olympus Mons and Bealty[25] reported a volume of $3–4 \times 10^6$ km^3. From the new map (Fig. 1) we calculate a total volume of 2.594×10^6 km^3 for the part of the volcano above the 5-km elevation. Assuming a density of 3.0 g cm^{-3} (ref. 26), we estimate the topographic mass of Olympus Mons to be 7.782×10^{18} kg above the 5-km elevation. If estimated volumes between elevations of 2 and 5 km are added to this calculation, the total volume above the 2-km contour is 3.862×10^6 km^3. This, in turn, gives a total mass of 11.586×10^{18} kg above the 2-km elevation.

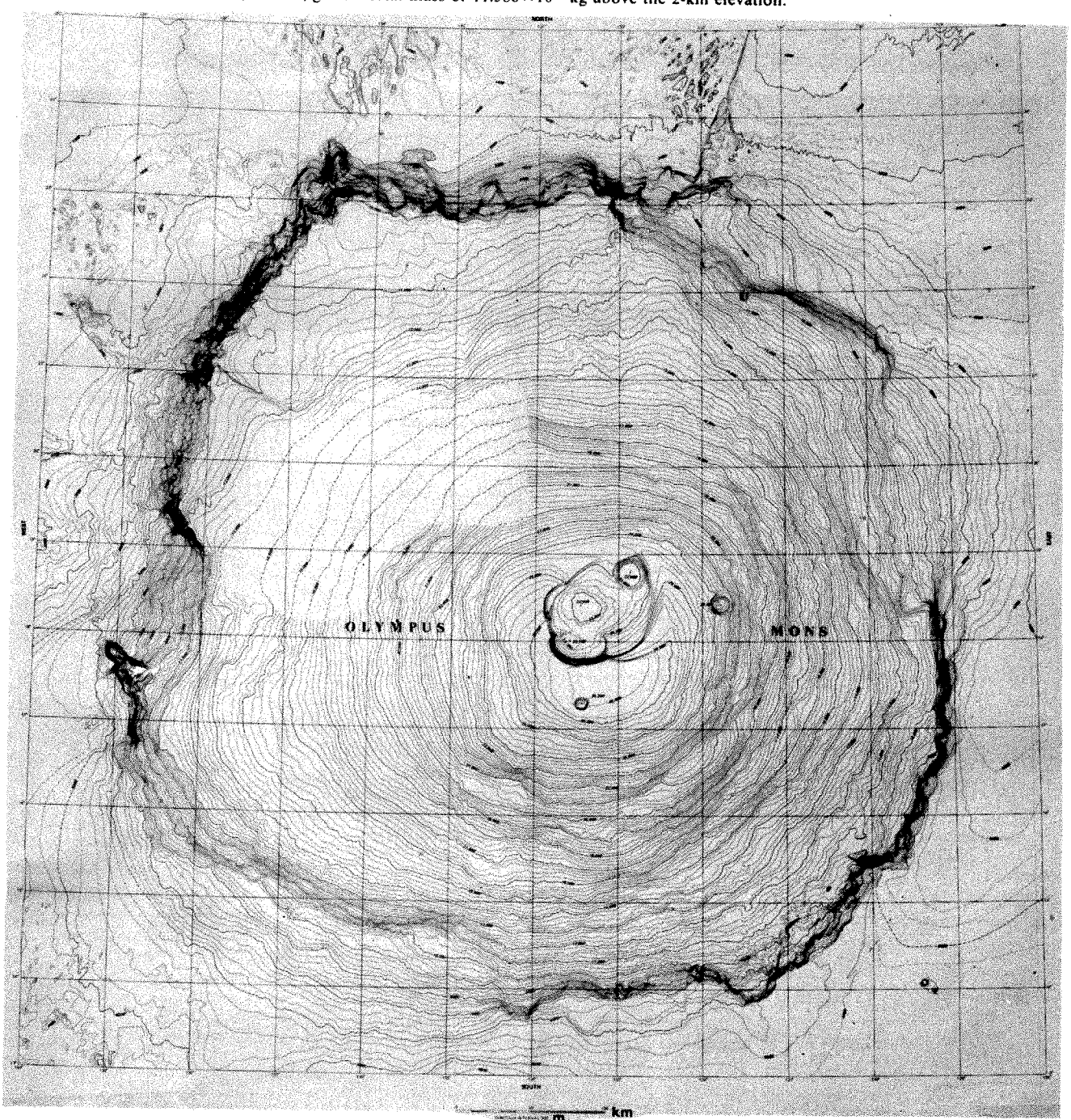


Fig. 1 Topographic map of Olympus Mons. Scale of the map, 1:1,000,000; contour interval, 200 m. Contours and elevations are in metres and are referred to the Mars topographic datum. A transverse Mercator projection was used with the central meridian placed at 134 °W long

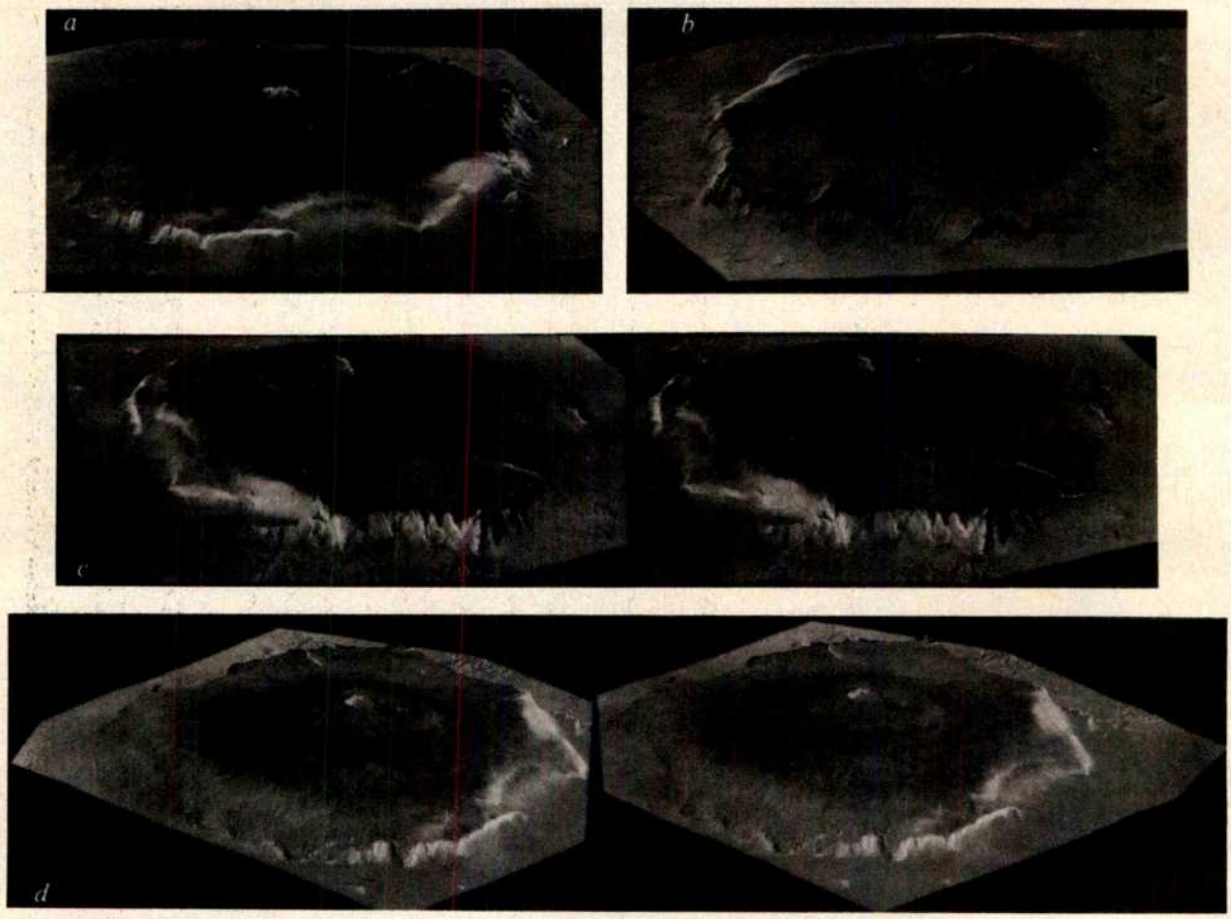


Fig. 2 Perspective views of Olympus Mons. Pictures were generated by digital image processing, from Viking Orbiter photograph646A28. Projection is controlled by digital elevation data collected at the time of the map compilation (Fig. 1). Viewing angle is 25° from the horizon. Vertical exaggeration, ×5. *a*, *b*, Monoscopic views from due east and due west; *c* and *d* are stereoscopic views from due north and from the south-east, respectively. Both stereo pairs have 5° separation in azimuth between the left and the right pictures. A stereoscope may be used for stereoscopic viewing.

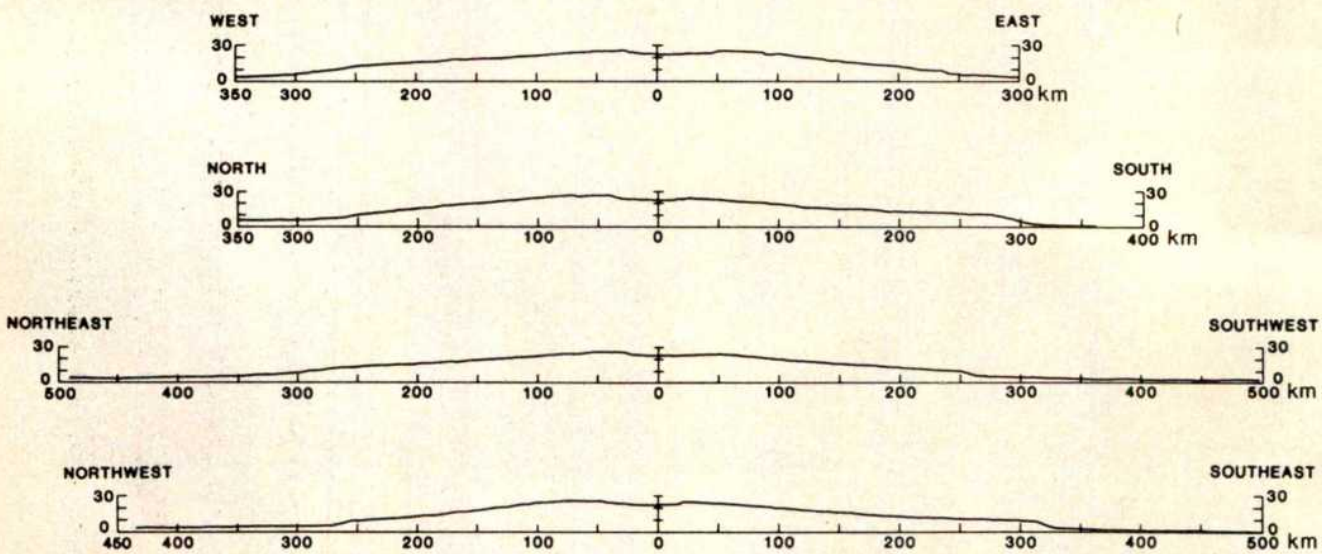


Fig. 3 Profiles measured from the topographic map (Fig. 1) of Olympus Mons. All are drawn so that they pass through the approximate centre of the summit caldera at 18.4° N lat. and 133.45° W long.

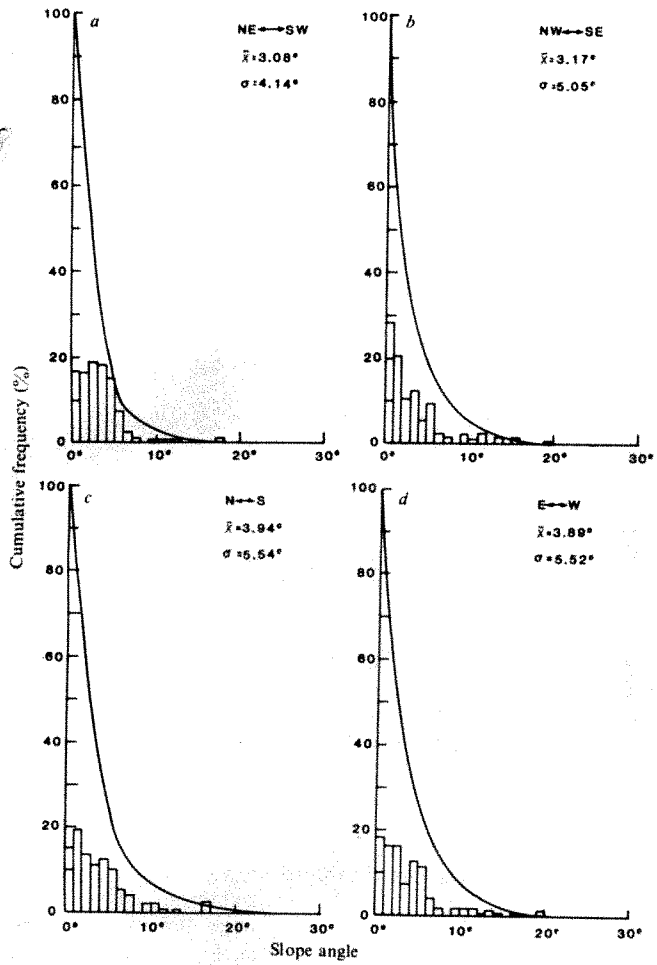


Fig. 4 Slope–frequency distributions of Olympus Mons flanks. Bars represent percentage of samples, by 1° increments of slope angle. Curved lines indicate cumulative frequency of slope angle. The quantity x is the mean absolute slope and σ is the algebraic standard distribution of the slope. *a–d*, Slopes measured along NE–SW, NW–SE, N–S and E–W directions respectively. The slope base is 5 km. These profiles, which were derived using a method of terrain analysis employing slope–frequency distributions[23,24], show the mean absolute slopes measured along the NE–SW, NW–SE, N–S and E–W directions, to be 3.08°, 3.17°, 3.94° and 3.89°, respectively. The algebraic standard slopes are 4.14°, 5.05°, 5.54° and 5.52°. Although the flank surface appears slightly smoother along its NE–SW direction, the surface of Olympus Mons shield is generally rough.

above the plains. Beyond the scarp, curvilinear troughs and ridges form a grooved terrain, the aureole deposits, which surround Olympus Mons. These deposits are some of the most puzzling features of the Olympus Mons region[1,21].

Figure 3 shows profiles measured from the map along the east–west, north–south, north-east–south-west and south-east–north-west directions. Slopes are divided, by elevation, into three groups. Within the 2–7-km interval, slopes of 2°–3° prevail; slopes of 8°–24°, between 7 and 16 km, are steeper mainly because there are several steep scarps within this elevation range; upper slopes, from 13 km to the outside rim of the caldera at 24 km (refs 2,22), measure 2.5°–6.5°.

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Can X-ray diffraction distinguish between protium and deuterium atoms?

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Isotopes are not normally considered to be distinguishable by X-ray crystal structure analysis. There is, of course, no problem as far as neutron diffraction is concerned, as neutron scattering amplitudes vary markedly and irregularly from one nuclide to another[1]. In particular, the neutron scattering amplitudes of protium and deuterium have opposite signs so that these two isotopes are especially easily identified. Indeed, in a key experiment nearly 20 yr ago, this difference was used together with the anomalous neutron scattering of ^{6}Li to establish the absolute configuration of an enzymatically formed α-monodeuterioglycolic acid[2]. We discuss here the possibility of distinguishing deuterium from protium by X-ray diffraction on the basis of the difference in the vibrational behaviour of the two isotopic species.

Least-squares analysis of sufficiently accurate and extensive diffraction data provides information not only about the atomic positions but also about the vibrational behaviour. This information is usually expressed as the components of a tensor $\mathbf{U}_i$ for each atom, whereby the product $\mathbf{l}^T\mathbf{U}_i\mathbf{l}$ is the mean-square vibrational amplitude of the atom i about its equilibrium position in the direction of the unit vector $\mathbf{l}$ (ref. 3). For light X-ray scatterers, such as hydrogen atoms, the tensors $\mathbf{U}_i$ are usually contracted into scalars U_i (isotropic vibrational parameters) which represent the spherically-averaged motion.

Examination of the results of recent low-temperature X-ray crystal structure studies indicates that isotropic U values (mean-square vibrational amplitudes) of hydrogen atoms in small to medium-sized organic molecules are typically in the range 150–300×10^{-4} Å^2 at temperatures around 100 K (refs 4–10). They are usually larger than equivalent isotropic U values of the heavier atoms, partly because of the effect of internal molecular motions and partly because the hydrogen atoms tend to be situated on the outside of the molecules and hence further from the molecular rigid-body librational axes. The corresponding reported standard deviations of U_H (as derived by least-squares analysis) are typically ~30–40×10^{-4} Å^2 for reasonably good quality 100 K measurements and are perhaps as low as, say, 15–20×10^{-4} Å^2 for data sets of the highest quality available.

This is about the precision level that would be required to distinguish isotropic U of protium from that of deuterium at the 2σ (98% probability) level. For example, from tables of

Table 1 Isotropic U values ($\times 10^4$) for hydrogen atoms obtained by least-square analysis from data sets I, II and III with different weighting schemes

	$w = 1$		$w = 1/\sigma^2(F_o)$		$w = 1$	
Data set	U (Å^2)	d(X–H) (Å)	U (Å^2)	d(X–H) (Å)	U (Å)^2	d(X–H) (Å)
H(21) I	98 (30)	0.996 (13)				
II	105 (24)	0.994 (11)	95 (20)	0.977 (9)	120 (27)	1.09
III	84 (25)	0.991 (12)	72 (20)	0.959 (9)	105 (28)	1.09
H(31)	188 (35)	0.985 (15)				
	193 (29)	1.004 (12)	194 (24)	0.940 (10)	224 (34)	1.09
	190 (32)	1.009 (12)	176 (25)	0.936 (11)	221 (36)	1.09
H(32)	224 (38)	0.999 (15)				
	239 (32)	1.022 (12)	175 (23)	0.944 (10)	264 (36)	1.09
	191 (33)	1.030 (12)	141 (24)	0.951 (10)	212 (36)	1.09
H(41)	237 (39)	0.976 (16)				
	242 (32)	0.961 (13)	313 (30)	1.003 (12)	361 (42)	1.05
	267 (37)	0.966 (14)	291 (31)	1.029 (12)	383 (47)	1.05
H(42)	344 (47)	0.929 (18)				
	315 (37)	0.908 (15)	394 (33)	0.808 (13)	443 (49)	1.05
	303 (40)	0.919 (16)	394 (35)	0.827 (14)	408 (49)	1.05
H(43)	285 (43)	0.910 (16)				
	292 (36)	0.859 (14)	356 (32)	0.940 (12)	497 (51)	1.05
	334 (42)	0.882 (15)	362 (35)	0.983 (13)	495 (54)	1.05
H(44)	304 (44)	0.920 (17)				
	225 (32)	0.915 (13)	224 (26)	0.944 (11)	379 (44)	1.05
	266 (38)	0.922 (14)	273 (30)	0.982 (12)	409 (49)	1.05

Bond distances obtained from the same calculations and involving the hydrogen atoms are also given. Estimated standard deviations are in parentheses.

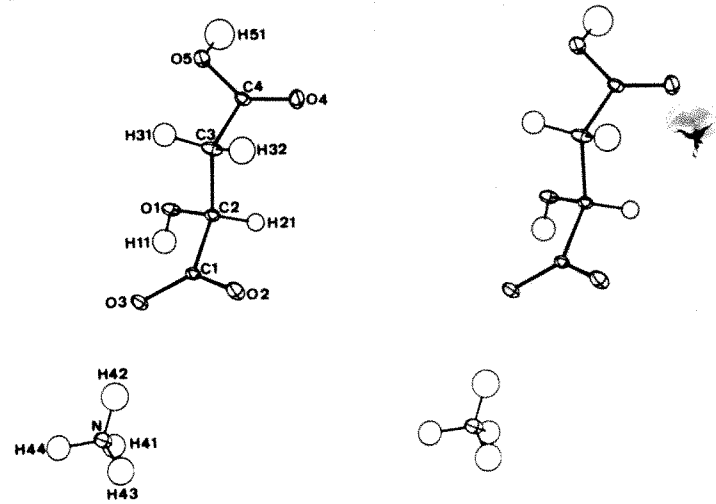


Fig. 1 Stereo view of molecule, showing atomic numbering. Vibrational ellipsoids are based on data set II and are drawn at the 50% probability level[21].

molecular vibrational frequencies for propane and 2,2-D_2-propane[11], the equivalent isotropic U_H and U_D can be estimated to be 119×10^{-4} Å for the CH_2 group and 84×10^{-4} Å^2 for the CD_2 group at 100 K. The difference of 35×10^{-4} Å^2 should be detectable by X-ray diffraction even if the presence of various systematic errors in this method could raise questions about the absolute level of the U_H values obtained.

We decided to try to determine by X-ray analysis the relative configuration of the two stereogenic centres in deuterium-labelled malic acid obtained by enzymatic addition of D_2O to fumaric acid. Although the answer to this stereochemical question was given more than 20 yr ago[12,13], we took the problem up again for methodological reasons, not because of any doubts about the earlier conclusion. Meanwhile an independent confirmation has been provided by a neutron diffraction analysis of the crystalline (+) (*R*)-α-phenylethyl ammonium salt of 2*S*-3-D-malate[14] prepared enzymatically from fumaric acid in a similar way as in the present study. Malic acid itself does not seem to yield suitable well-formed crystals, and we finally settled on the mono-ammonium salt[15] as the best candidate for an accurate low-temperature X-ray diffraction study, a choice that in retrospect may not have been optimal for reasons discussed below.

First, to test whether standard deviations of the structural parameters from such an experiment were reproducible, we measured two independent low-temperature data sets for the undeuterated compound: different crystal specimens, different diffractometers, different low-temperature devices, slightly different temperatures (~95 K), and different limiting reflection spheres. We refer to these as data sets I (extending to sin $\theta/\lambda = 0.90$ Å^{-1} at 93 K) and II (extending to sin $\theta/\lambda = 1.19$ Å^{-1} at 95 K).

The results obtained with these two data sets do not differ in any important respect. As far as the heavy atoms are concerned, atomic positions agree within a few 10^{-4} Å and vibration parameters within a few 10^{-4} Å^2. The hydrogen positions and isotropic U values are sensitive to the scattering factor and weighting system used in the least-squares analyses. Indeed, as Table 1 shows, the variations in the U_H values produced by changing the weighting system within data set II are as large as, or sometimes even larger than, the differences between values obtained from the two data sets using the same (unit) weights. For atom numbering see Fig. 1. The hydroxyl and carboxyl hydrogens, which are engaged in strong hydrogen bonds, are less well defined than the others and have much larger U_H values in both analyses. The ammonium hydrogens, which are also engaged in hydrogen bonds, have larger U_H values, presumably because of rigid-body librational motion of the ammonium group. For the three hydrogen atoms attached to carbon, there is nearly perfect agreement between the U_H values obtained from the two data sets using the same (unit) weights (see Table 1).

This comparison encouraged us to measure diffraction data for a crystal of the mono-ammonium salt of a malic acid sample prepared by enzymatic addition of D_2O to fumaric acid by means of a fumarase preparation from pig heart (Böehringer, Mannheim). Our target was the determination of the relative configuration of the two stereogenic centres, the traditional one at C(2) bearing the hydroxyl group and the new one resulting from isotopic substitution at C(3).

Considerable pains were taken to ensure that the diffraction data for the deuterated crystal (data set III) were measured as closely as possible in the same conditions as data set II for the undeuterated compound. Indeed, the two sets of diffraction measurements including unit-cell dimensions and their temperature dependence are so similar that they could easily be taken for duplicate sets of measurements for the same compound. A parallel, almost perfect correspondence holds for the positional and vibrational parameters of the heavy atoms derived from the two data sets with matching weights (Table 2). In particular, the U_{ij} components of the heavy-atom vibration tensors agree to within a few 10^{-4} Å^2 units. However, some of the differences, although small, are systematic. First, the *b* axis of the deuterated crystal is measurably (~0.01 Å) longer than that of the undeuterated crystal. This is the direction with the largest coefficient of thermal expansion and is perpendicular to the well-defined layers of molecules that constitute the crystal structure. Table 2 shows that a corresponding increase in the mean-square vibrational amplitudes U_{22} of the heavy atoms in this direction is just detectable.

The U_H values obtained from data sets II and III show greater variation but they could still also be taken as belonging to the same compound. However, given that one of the two methene atoms is protium and one deuterium, the heavier isotope can be identified as H(32) with high probability. On going from data set II to data set III this atom shows a decrease in U_H amounting to $30–50 \times 10^{-4}$ Å^2, depending on the weighting system, whereas its partner H(31) shows nearly constant behaviour. As far as the other hydrogen atoms in this comparison are concerned, some show a slight decrease, some an increase, and some erratic behaviour. Given that the configuration at C(2) is *S*, the one at C(3) is then assigned as *R*, in agreement with previous conclusions[12–14].

Unfortunately, the standard deviations of the vibration parameters are ~50% larger than we had hoped to achieve. One

Table 2 Positional parameters (for C, N and O atoms $\times 10^5$, for H atoms $\times 10^4$) and vibrational parameters (all $\times 10^4$) for mono-ammonium 2*S*-malate (upper value) and 2*S*-3-D-malate (lower value) at 95 K

	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N II	92,965 (6)*	86,828 (8)	3,737 (5)	84 (1)	176 (2)	90 (1)	−2 (1)	4 (1)	−5 (1)
N III	92,932 (7)	86,810 (8)	3,736 (5)	85 (1)	175 (2)	91 (1)	−3 (1)	4 (1)	−6 (2)
C(1)	54,602 (6)	85,629 (7)	25,965 (5)	50 (1)	110 (2)	90 (1)	−3 (1)	1 (1)	−6 (1)
	54,590 (7)	85,630 (8)	25,977 (5)	51 (1)	111 (2)	89 (1)	−3 (1)	0 (1)	−5 (1)
C(2)	35,128 (6)	90,278 (7)	26,797 (5)	52 (1)	115 (2)	94 (1)	0 (1)	1 (1)	−2 (1)
	35,118 (7)	90,292 (8)	26,811 (5)	52 (1)	114 (2)	94 (2)	0 (1)	1 (1)	−2 (2)
C(3)	25,395 (6)	77,788 (8)	35,102 (6)	59 (1)	164 (2)	120 (2)	10 (1)	11 (1)	41 (2)
	25,401 (7)	77,835 (9)	35,151 (6)	60 (1)	165 (2)	120 (2)	11 (2)	11 (1)	40 (2)
C(4)	7,209 (6)	84,138 (8)	38,049 (5)	59 (1)	161 (2)	79 (1)	−3 (1)	3 (1)	5 (1)
	7,211 (7)	84,169 (9)	38,085 (5)	59 (1)	161 (2)	78 (1)	−4 (1)	3 (1)	5 (2)
O(1)	26,941 (5)	90,245 (6)	14,768 (4)	66 (1)	177 (2)	96 (1)	−3 (1)	−10 (1)	34 (1)
	26,911 (6)	90,215 (7)	14,784 (4)	66 (1)	177 (2)	96 (1)	−4 (1)	−11 (1)	33 (1)
O(2)	63,384 (5)	86,954 (7)	36,098 (4)	63 (1)	255 (2)	101 (1)	16 (1)	−17 (1)	−45 (1)
	63,382 (6)	86,986 (8)	36,106 (5)	63 (1)	258 (2)	102 (1)	17 (1)	−17 (1)	−46 (2)
O(3)	60,568 (5)	80,966 (6)	15,597 (4)	70 (1)	180 (2)	88 (1)	10 (1)	9 (1)	−17 (1)
	60,546 (6)	80,933 (7)	15,609 (4)	70 (1)	180 (2)	88 (1)	10 (1)	9 (1)	−18 (1)
O(4)	4,532 (6)	96,146 (7)	44,864 (5)	103 (1)	219 (2)	156 (2)	0 (1)	−2 (1)	−75 (2)
	4,519 (7)	96,141 (8)	44,917 (6)	103 (1)	221 (2)	156 (2)	0 (2)	−2 (1)	−76 (2)
O(5)	−5,342 (5)	75,681 (7)	32,471 (5)	62 (1)	207 (2)	142 (1)	−5 (1)	−8 (1)	−51 (1)
	−5,334 (6)	75,728 (8)	32,479 (5)	63 (1)	210 (2)	141 (2)	−5 (1)	−8 (1)	−51 (1)
	x	y	z	U		x	y	z	U
H(11)	3,404	9,584	833	443 (48)	H(42)	8,170	8,377	892	443 (49)
	3,392	9,555	817	495 (54)		8,158	8,362	878	408 (49)
H(21)	3,375	10,253	3,113	120 (27)	H(43)	10,403	8,852	948	497 (51)
	3,369	10,247	3,123	105 (28)		10,413	8,829	938	495 (54)
H(31)	2,423	6,594	3.014	224 (34)	H(44)	9,662	7,744	−264	379 (44)
	2,400	6,595	3.029	221 (36)		9,676	7,760	−274	409 (49)
H(32)	3,204	7,680	4,418	264 (36)	H(51)	−1,702	8,046	3,374	517 (53)
	3,210	7,662	4,420	212 (36)		−1,714	8,020	3,375	441 (51)
H(41)	9,031	9,731	−188	361 (42)					
	9,041	9,714	−206	383 (46)					

* Standard deviations are in parentheses.

reason for this is the markedly anisotropic character of the molecular translational vibration tensor **T**, due to the strongly layered nature of the crystal structure. For a given atom type, standard deviations of vibrational parameters are closely linearly dependent on the vibrational parameters themselves. Thus for the carbon atoms from data set II (unit weights, Table 2) $\sigma(U_{ii}) = 8.9 + 0.00908\ U_{ii}$ (units of 10^{-5} Å^2) and for the hydrogen atoms (unit weights, Table 1) $\sigma(U) = 17.2 + 0.00630\ U$ (units of 10^{-4} Å^2).

A general reduction of the U_H values would therefore have been very helpful in reducing $\sigma(U_H)$ towards the desired level. This is one reason for thinking that mono-ammonium malate, with its strongly layered crystal structure, may not have been an ideal choice for our experiment. It is also unfortunate that the methene carbon C(3) is the one that shows the largest vibrational motion, a motion that is unavoidably transmitted to the hydrogens it carries. Nevertheless, our experiment suffices to show that with present measurement techniques, the distinction by X-ray diffraction between protium and deuterium atoms in a medium-sized organic molecule is just possible.

Even with large and accurately measured data sets, the X-ray method cannot be expected to match neutron diffraction in its ability to distinguish between protium and deuterium. However, the much wider availability of the X-ray method calls for a critical examination of its potentialities in this direction. We therefore draw attention to several points that will have to be considered by anyone hoping to improve on our work.

First, the crystal chosen for study should have smaller, less anisotropic, vibrational parameters than the one we chose. A slight gain could have been achieved with our crystals by lowering the temperature, but probably not enough to make a significant improvement.

The use of a noncentrosymmetrical, chiral crystal for an experiment of this kind can hardly be avoided as the isotopically labelled, enantiomerically-pure compound must crystallize in a chiral arrangement. At most, crystals of such a compound could be pseudo-centrosymmetric. However, there are well known difficulties associated with the refinement of nearly centrosymmetric structures in noncentrosymmetric space groups[16]. From a symmetry breakdown involving merely a difference between U_H values of two hydrogen atoms, the chance of a successful resolution seems to be remote.

The weighting problem also merits more careful attention than we have devoted to it. The problem with 'normal' weights (inversely proportional to variances) is that too large a weight is assigned to the weak, low-order reflections, which are just the ones most affected by the insufficiency of the free-atom formalism. The contamination of the resulting atomic parameters by the deformation density is especially severe for the hydrogen atoms, leading to the characteristic displacement of these atoms towards their bonded partners and also to less easily recognizable errors in the vibration parameters. These errors in the hydrogen parameters reduce the value of the weighted residual $\Sigma\omega_i(\Delta F_i)^2$ and hence of the standard deviations as estimated from the least-squares analysis.

Refinements using only high-order reflections give positional and vibrational parameters that differ perceptibly from those obtained from full-data refinements[17]. Provided that the high-order data are sufficiently extensive and accurate, the so-derived parameters are less contaminated by the valency electron density and hence, in general, closer to the true values (that is, those obtainable from a neutron-diffraction experiment). High-order refinements on data sets II and III (HO, Table 3) led to heavy-atom parameters with estimated standard deviations less than half of those derived for the full-data refinements and listed in Table 2. The C(2)–H(21) distance refined to 1.08(2) and 1.10(3) Å respectively, that is, virtually to the correct value. The other C–H, N–H, and O–H bond distances still showed deviations (mostly shortenings) of 0.03–0.10 Å. Thus, the high-order reflections contain considerably more signal from H(21) with its appreciably lower U_H than from the other hydrogen atoms. In fact, as Table 1 shows, there is an evident correlation between apparent C–H bond length and U_H: the smaller the C–H distance, the smaller the U_H. This introduces an additional element of uncertainty into the determination of the latter quantities. One possibility is to constrain the C–H, N–H and O–H bond distances to their correct values. This leads to U_H values that are larger, have larger standard deviations, but are more uniform

Table 3 Experimental details for three sets of X-ray diffraction measurements on mono-ammonium 2*S*-malate (data sets I, II) and mono-ammonium 2*S*-3-D-malate (data set III)

Data set	I	II	II (HO)	III	III (HO)
Temperature (K)	93	95	95	95	95
sin θ/λ range (Å^{-1})	0–0.9	0–1.19	0.7–1.19	0–1.19	0.7–1.19
No. of observed reflections	2,280	5,097	3,996	5,106	4,006
No. of reflections with $I>3\sigma(I)$	1,882	3,977	2,929	3,888	2,829
No. of variables	128	128	128	128	128
Weight	1	1	$1/\sigma^2(F_0)$	1	$1/\sigma^2(F_0)$
$R(F)$	0.021	0.023	0.017	0.023	0.015
$R_w(F)$	0.024	0.029	0.017	0.032	0.015

between the two data sets II and III (except, of course, for H(32), which shows a drop of 50×10^{-4} Å^2). In retrospect, given that the hydrogen atoms H(31) and H(32) do not scatter significantly at high angles, our experimental effort might have been better spent by making two duplicate sets of measurements out to a lower sin θ/λ limit, say, 0.9 Å^{-1}.

Another question concerns the possibility of measuring the components of the anisotropic tensor $\mathbf{U}_H$ instead of just the contracted scalar U_H, for the vibrational amplitudes of the hydrogen atoms can be expected to be far from isotropic. The difficulties are formidable, and no attempt to accomplish this from X-ray diffraction data has been convincing.

Finally, there is the question of whether anything might be gained by expressing the static deformation density in parametric form, and refining the additional parameters together with the usual positional and vibrational parameters by the least-squares method. If sufficient data were available, the decrease in the weighted residual could more than compensate for the increase in the number of parameters, leading to a lowering in the standard deviations of the positional and vibrational parameters.

The X-ray measurements were carried out on two different Enraf-Nonius CAD4 diffractometers each equipped with a graphite monochromator (Mo$K\alpha$ radiation, $\lambda=0.7107$ Å) and a modified Enraf-Nonius gas-stream low-temperature device. Temperature fluctuations monitored by a thermocouple for data set I and by a PT100Ω resistor for data sets II and III were less than ±0.5 K during the experiments.

The two compounds were recrystallized by slow evaporation of an aqueous solution at about 293 K. Different crystal specimens were used for data sets I and II (space group $P2_12_12_1$). Unit-cell dimensions at 95 K were obtained by least-squares refinement of setting angles for 24 reflections with 2θ values in the range 96° to 105°: data set II, $a=7.6386(7)$, $b=8.0254(7)$, $c=10.5873(8)$ Å; data set III, $a=7.6366(6)$, $b=8.0351(8)$, $c=10.5840(8)$ Å. Integrated relative intensities were obtained by $\omega, 2\theta$ scans.

The room-temperature coordinates[15] served as starting point for our least-squares refinements (X-ray-system[18]). Final positional and vibrational parameters, listed in Table 2, are based on full-matrix least-squares analyses using scattering factors for neutral atoms[19,20] and including an isotropic extinction correction. Additional experimental details for the three data sets are summarized in Table 3.

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Microgranitoid enclaves in granites—globules of hybrid magma quenched in a plutonic environment

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Microgranitoid enclaves (autoliths, cognate xenoliths, mafic inclusions) are common in high-level granitoid plutons. They have relatively fine-grained igneous (microgranitoid) microstructures[1], and many show evidence of having flowed in a magmatic condition[2]. These features counter interpretations that the enclaves represent transformed solid fragments of diverse wall rocks[3–5] or restite[6–11]. An alternative interpretation is that the enclaves represent globules of mafic magma that have mingled ('commingled') and quenched in the granitoid host magma[12–16]. A detailed review of the literature, occurrence, morphology and composition of microgranitoid enclaves strongly supports this view, but indicates that the enclave magmas range in composition from mafic to felsic, and that magma-mixing may be involved in their formation[17]. I summarize here the main features of microgranitoid enclaves and briefly outline the possible processes leading to their incorporation as magma globules in the host granitoid.

The microgranitoid enclaves occur in both I-type and S-type high-level granitoid plutons[17,18]. Their abundance is not related to pluton contacts nor the number of hornfels xenoliths[2], and they are typically much more abundant than hornfels or other xenoliths[17]. Their distribution may be locally uniform, but is commonly irregular, and they may be concentrated in swarms[2]. They are generally unrepresented among the exposed country rocks[6].

The microgranitoid enclaves typically have rounded to ovoid shapes[1,2], though sub-angular and, more rarely, angular enclaves may be present[2]. Some have cuspate margins, with lobes convex towards the host granitoid. Elongate enclaves are markedly ellipsoidal to lenticular, and are aligned in areas of the granitoid that show a prominent primary flow foliation[2], especially near the margins of the pluton[7]. Their alignment and degree of elongation correlate with the intensity of the flow foliation in the host granitoid[2,17]. Neither enclave nor granitoid show microstructural evidence of crystal plasticity or recrystallization, so that the extension appears to have been accomplished by relative movement of crystals and melt; that is, when they were in a magmatic condition[17,19].

The enclaves generally have uniform, random microstructures, though some have finer-grained margins. Some have flow alignment of plagioclase or hornblende crystals, which may be parallel to the enclave margins and may be deflected around phenocrysts. The enclave microstructures are typical of fine- to medium-grained igneous rocks, consisting commonly of euhedral phenocrysts of zoned plagioclase, with or without quartz, biotite, hornblende (in I-types) or orthopyroxene ± cordierite (in

S-types), set in a groundmass rich in biotite and euhedral laths of zoned plagioclase, commonly with interstitial to poikilitic quartz, and/or K-feldspar[17]. They typically contain apatite needles, indicative of magma quenching[20]. They have compositionally and microstructurally similar counterparts in calc-alkaline volcanic rocks[13,14,21,22]; many of these contain interstitial glass and some have skeletal, zoned crystals of hornblende and plagioclase, indicating a magmatic condition[13,14,22].

The enclaves are darker and finer-grained than their host granitoids, but although some are mafic[16], most are intermediate to silicic[23]. Many compositional varieties may occur together. The mineral assemblages in the enclaves are generally the same as those in the host granitoid, but the proportions and compositions of the minerals are typically different[2,17].

Magmatic orthopyroxene + cordierite + plagioclase in the microgranitoid enclaves of the S-type Cowra Granodiorite, New South Wales, Australia[18], indicate crystallization at relatively low confining pressures (100–200 MPa)[24]. If these enclaves are representative, microgranitoid enclaves appear to crystallize at pluton emplacement levels, which agrees with the observation that they are restricted to relatively high-level plutons[25].

The inference that the enclaves were globules of magma at the time that the flow occurred can explain the typical rounded to cuspate shapes of the enclaves. The rounded shapes appear to be primary, rather than due to rounding of angular, solid fragments, because nearby hornfels xenoliths generally show little or no rounding. The more angular enclaves could be fragments of solid, quenched igneous rocks, but they could also be magma fragments, because magma may break (like pitch) under rapidly applied stress[26].

The relatively fine-grained microstructures indicate relatively rapid crystallization; that is, quenching of magma in the plutonic environment. Examples of mafic (mafic to intermediate) magma quenched against felsic magma are common in volcanic[22,26–28], subvolcanic[12,26,29–31] and plutonic[16,19,29,32–37] environments, as discussed in detail elsewhere[17]. The process involves magma mingling, whereby rounded to cuspate globules and streaks of the more mafic magma become dispersed through the felsic magma. The more mafic magma crystallizes more rapidly (forming finer-grained aggregates) than the felsic magma, because it is more undercooled than the felsic magma[22], once thermal equilibrium between the two has been established. Mafic enclave magmas would be much more strongly undercooled than their graintoid host magmas[22]. However, even where the enclave and granitoid magmas are fairly similar in composition, the more mafic enclave magma can crystallize more rapidly, because small differences in the degree of undercooling can give rise to marked increases in nucleation rate and marked decreases in growth rate[17].

The origin of the magma that produced the enclaves is a matter for speculation, as examples of magma mingling show only that magmas can intimately coexist, not how they originated[11]. Though felsic and more mafic calc-alkaline magmas are chemically miscible[37–40], they can remain separate[38], particularly if small volumes of the more mafic magma are enclosed in large volumes of felsic magma and if stirring is minimal[39,40], because chilling increases the viscosity of the more mafic globules, as well as promoting rapid crystallization.

Enclave magmas conceivably could be derived from: (1) partly crystallized, quenched margins of the granitoid[18,41] (if this can account for the evidence of hybridism and the observed compositional range of enclaves in a single pluton), (2) repeated mingling of a variety of more mafic (?)hybrid magmas injected into, or fortuitously encountered by, the granitoid, or (3) a more mafic lower layer in the pluton that is partly mixed with the granitoid magma[17]. Careful isotopic studies could conceivably indicate whether the enclave magmas are closely related to the granitoid magma[42] (for example, either differentiates of it or separate batches of (?)hybrid magma from the same or an isotopically similar source) or whether they involve contributions from unrelated magmas[22].

One way of producing the observed compositional variation of the enclaves is by magma mixing, which involves homogenization of the two melts (in contrast to magma mingling). The common presence of plagioclase with resorbed or dendritic cores, K-feldspar megacrysts with oligoclase rims, and rounded quartz xenocrysts with fine-grained mafic rims indicates that many enclave magmas are related in origin to rocks showing mineralogical evidence of hybridism[17,28,29], for example, the Skye marscoite, for which magma mixing is commonly postulated[29,43]. Therefore, the enclave magmas may be products of magma mixing[15], either in the same pluton[17], or at a site removed from the actual magma mingling in the pluton, as commonly appears to be so for clear examples of magma mingling[29,30].

Reid *et al.*[16] recently presented clear evidence of the mingling of felsic magma with more mafic magma globules in the Sierra Nevada, California, adding that solution and disintegration of the resulting enclaves contaminated the surrounding granitoid. However, in my experience, microgranitoid enclaves typically show little or no evidence of disintegration, and the surrounding granitoid is generally not obviously modified[36]. Furthermore, disintegration of enclaves should disseminate finer-grained material than is typical of granitoids. The tendency of enclaves to be more abundant in more mafic granitoids[8,16] may be due simply to local accumulation of enclaves and precipitated crystals[44]. The common coexistence of enclaves of variable composition[17] and the microstructural evidence of hybridism suggest that the compositional variety of the enclave magmas was produced away from the exposed site of final mingling. Furthermore, reports of 'lamprophyric' dykes with corroded xenocrysts, resembling enclaves in adjacent granitoids[29,45], suggest that enclave magmas can exist either independently of, or as a separate layer in, their host granitoid magma bodies.

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Inverse relationship between Sr isotope diversity and rate of oceanic volcanism has implications for mantle heterogeneity

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Cohen and O'Nions[1] noted that basalts from the Mid-Atlantic Ridge (MAR) display greater diversity of Pb, Nd and Sr isotopes than do basalts from the East Pacific Rise (EPR). They attributed this difference not to greater isotopic heterogeneity beneath the MAR, but rather to more effective mixing (to eradicate heterogeneity) beneath the EPR. Allègre *et al.*[2] reached the same conclusion on the basis of isotope data including noble gas isotopic data. To test this idea further, I compare here the average spreading rate of nine portions of the mid-ocean ridge system with the measured $^{87}Sr/^{86}Sr$ diversity of basalts. This shows that the maximum observed $^{87}Sr/^{86}Sr$ diversity is inversely proportional to spreading rate. Furthermore, globally averaged eruption rates for mantle-derived rocks of intra-oceanic island arcs, ocean islands and the mid-ocean ridge system exhibit a negative correlation with $^{87}Sr/^{86}Sr$ diversity. These results suggest that the upper mantle is everywhere heterogeneous on a small scale and that the extent of observed heterogeneity is a function of mixing. Independent geophysical and petrological evidence for the existence of steady-state crustal magma chambers beneath fast-spreading ridges and their absence below slow spreading ridges favours this hypothesis because mixing is enhanced by the presence of a magma chamber. Alternatively, mixing during melt production in the mantle, during melt segregation or during ascent could also result in the observed patterns if the degree of mixing is related to eruption rate. Large heterogeneities (mantle plumes?) are required to explain why isotopic variations are well correlated geographically in some places but not in others.

Several workers have suggested that the sub-oceanic mantle source for basalt melts is ubiquitously heterogeneous on a small scale[3–6]. A 'plum pudding' mantle of this type can, on melting, produce suites of basalts with variable $^{87}Sr/^{86}Sr$. The variability depends partly on the sizes of the isotopically heterogeneous regions compared with the size of the region undergoing batch melting. Batch melting of regions that are large compared with the sizes of isotopically heterogeneous domains will produce a homogeneous melt with isotopic characteristics that approach those of the bulk source. On the other hand, batch melting of smaller regions produces melts that selectively sample the heterogeneities. Melting of several such small isolated regions would produce a suite of melts with diverse isotopic characteristics that, as a group, reflect the isotopic diversity of the source rather than its average bulk characteristics.

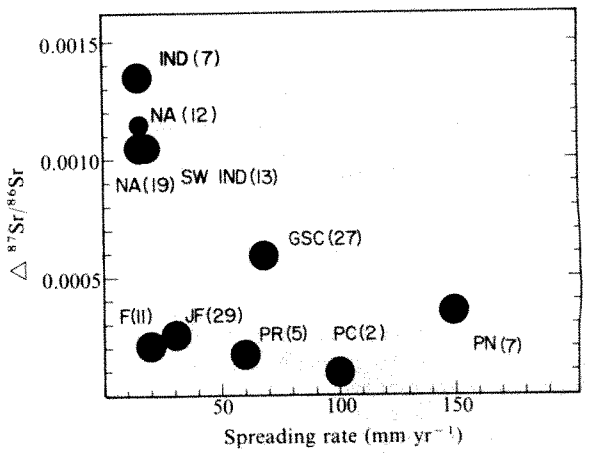


Fig. 1 Plot of $^{87}Sr/^{86}Sr$ range (Δ) against spreading rate for the data of Table 1. Numbers in parentheses are the number of samples. IND, Indian Ridge; NA, North Atlantic; SW IND, south-west Indian Ocean Ridge; F, FAMOUS Area; JF, Juan de Fuca Ridge; GSC, Galapagos Spreading Centre; PR, Pacific-Rivera (21°N); PC, Pacific–Cocos and PN, Pacific Nazca Ridge. For available high-precision analyses of glasses and carefully leached samples, there is an inverse correlation between the maximum strontium isotope diversity of MORB and the spreading rate.

Likewise, if the size of the melt region is held constant, melts produced by batch melting of plum pudding mantles would be expected to have isotopic characteristics that vary as a function of the extent of partial melting[4,7,8]. Small degrees of partial melting would selectively sample mangle domains having lower solidus temperature because of enrichments in volatiles, large ionic lithophile (LIL) elements and ^{87}Sr and larger extents of melting would result in dilution of this melt fraction with melt from more refractory LIL-poor mantle domains[9,10]. Suites of isotopically diverse melts could be produced in this way by lateral, depth or temporal variations in the extent of partial melting.

Isotopically diverse melts that are generated simultaneously in neighbouring regions of a plum pudding mantle by either variable extents of batch melting or variable size of melt region or both, may ascent and erupt without mixing to produce isotopi-

Table 1 Sr-isotopic, spreading rate and eruption rate data

Location	Highest $^{87}Sr/^{86}Sr$	Lowest $^{87}Sr/^{86}Sr$	Δ $^{87}Sr/^{86}Sr$	Data sources	No. of samples	Spreading rate (mm yr^{-1})
Indian Ocean	0.70397 ± 9	0.70262 ± 3	0.00135	42	7	10–20
SW Indian Ridge	0.70372 ± 6	0.70267 ± 4	0.00105	17	13	17.2
North Atlantic	0.70357	0.70251	0.00106	15	19*	10–20
North Atlantic	0.70346 ± 4	0.70231 ± 6	0.00115	16	12	10–20
Juan de Fuca Ridge	0.70263 ± 6	0.70237 ± 6	0.00026	14	29	30
Pacific–Nazca Ridge	0.70287 ± 5	0.70251 ± 5	0.00036	1, 43, 44	7	150
Pacific–21° N	0.70264 ± 8	0.70247 ± 6	0.00017	45	5	60
FAMOUS Rift	0.70296 ± 6	0.70276 ± 11	0.00020	45	11	20
Galapagos Spreading Centre	0.70307 ± 3	0.70248 ± 4	0.00059	46	27	65–70
Pacific–Cocos	0.70259 ± 3	0.70250 ± 4	0.00009	4	2	100
						Eruption rate† (km^3 yr^{-1})
Mid Ocean Ridges—compilation			0.0012	7	—	22
Ocean Islands—compilation			0.0042	7	—	1.5
Intra-Oceanic Island Arcs—compilation			0.0029	7	—	3.0

* Mean analyses.
† From ref. 18.

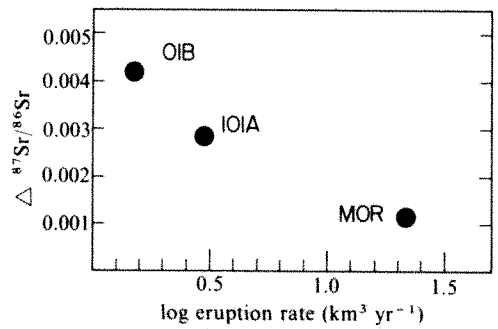


Fig. 2 Plot of $^{87}Sr^{86}Sr$ range (Δ) against global, time-averaged eruption rate for mid-ocean ridges (MOR), ocean islands (OIB) and intra-oceanic island arcs (101A)[18] Sr-isotope compilations are from ref. 7.

cally diverse lava suites. Alternatively, mixing of these diverse batches in the mantle or crust could partly or completely homogenize the diverse melts.

Table 1 is a compilation of published ranges of high-precision $^{87}Sr/^{86}Sr$ data for mid-ocean ridge basalts (MORB) with corresponding spreading rates[11–14]. These are measured values for fresh glasses and carefully leached crystalline basalts. Figure 1 shows that the maximum observed range of $^{87}Sr/^{86}Sr$ is inversely correlated with spreading rate for MORB; smaller ranges of variation are possible for smaller geographical subsets or may be artificially created by insufficient sampling. Ideally, of course, this sort of comparison is most meaningful if normalized to equal ridge crest lengths that are sampled with uniform density. Unfortunately, the available data are too fragmentary for this, or for rigorous statistical analysis of the variation along all ridge crest segments. Note that Fig. 1 contains data for ridge crest segments that display coherent geographical patterns of isotope variation (like the northern MAR[15,16]) as well as slow-spreading ridges that do not (like the south-west Indian Ocean Ridge.[17])

Table 1 also gives $^{87}Sr/^{86}Sr$ data compiled by Morris and Hart[7] for mantle-drived intra-oceanic island arcs, oceanic islands and the mid-ocean ridge system together with globally-integrated average eruption rate estimates of Crisp[18]. For these data, average eruption rate is inversely correlated with diversity of $^{87}Sr/^{86}Sr$ (Fig. 2). This agrees with the MORB data if the eruption rate at mid-ocean ridges is proportional to spreading rate.

Future additional sampling and analyses will be required to determine whether the patterns of Fig. 1 hold up or are merely artefacts. For future sampling of ridge crests it is important to distinguish between the axes of normal ridge crests and small, isotopically diverse seamounts that may be present near the ridge[8]. In addition, recently discovered petrological effects at ridge transform intersections[19–21] may be important, so it is crucial to know the locations of transforms and ridge off-sets[22,23].

If these general patterns are confirmed, there are several possible explanations for their existence. It is possible, of course, that the mantle sources of slow-spreading ridges are simply more heterogeneous than the sources below fast-spreading ridges. Alternatively, the extent of contamination of basalt magma may be related to spreading rate[24]. These seem unlikely, however, and I prefer to interpret these patterns in terms of melting of a plum pudding mantle associated with or followed by varying amounts of homogenization caused by mixing. This is reasonable in view of the large amount of independent geophysical and petrological evidence for the spreading rate dependence of steady state crustal magma chambers beneath ridge axes[25–29]. It is also possible, of course, that mixing takes place in the mantle during melting[30–32], segregation[33–35] or ascent[36]. Evaluation of this possibility must await better understanding of the thermal structure beneath ridges, their temporal variability and possible feed back and buffering of the thermal and rheologic properties of the mantle by melting and magmatic ascent processes[37–39]. If our preferred interpretation of the pattern in Figs 1 and 2 is correct, it has important implications for the nature and size of mantle reservoirs, their convective mixing[40,41] and their thermal and differentiation histories.

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Particulate and dissolved vanadium in the North Pacific Ocean

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The distributions of dissolved trace elements in the oceans result from a complex series of biogeochemical processes. Historically, most models describing the cycles of these elements have been formed around high quality data for the distributions of the dissolved elements[1]. I report here a detailed vertical profile of particulate vanadium and estimate its particle-associated flux. This flux suggests a surface depletion of dissolved vanadium which is verified by the first detailed determinations of dissolved vanadium in several profiles from the Pacific Ocean.

Particulate matter was collected from the upper 1,500 m of the Panama Basin by a large volume filtration system (LVFS)[2]. The samples were collected in July 1976 and again in March 1979 from the same location, 0° 44′N, 86° 10′W. The chemistry, biology, and vertical flux of material in the 1976 samples have been reported elsewhere[3] and a discussion of the 1979 samples is in preparation. Samples for dissolved vanadium analysis were collected from this site and several other locations in the North Pacific Ocean (Table 1).

The concentration of particulate vanadium in the >53 μm particles is shown in Fig. 1. The basic form of these two vertical profiles is driven by the distribution of total particulate mass[3]. The strong surface maximum is associated with the primary production of biogenic particulate matter. However, terrigenous material is also an important carrier of particulate vanadium. The compositions of fine suspended particulate matter[4] and material from sediment traps in the deep Atlantic[5] show excellent correlations between vanadium and aluminium at crustal ratios. Although there was significantly more biogenic and terrigenous particulate matter in the water column during March 1979, the concentration of particulate vanadium was higher during July 1976. The V/Al ratio in the surface sample from the 1976 Galapagos profile was 2.4×10^{-2}, which is more than one order of magnitude higher than the crustal ratio (8.5×10^{-4}). The ratio in the deeper samples dropped rapidly towards the crustal value as the biogenic material was remineralized. The same ratio in the March 1979 samples was near to the crustal value throughout the water column. The V/Al ratios in particles collected from sediment traps in the eastern tropical Pacific and from the underlying sediments (J. Dymond and R. C. unpublished data) are also near the crustal values. The flux to these traps (~0.7 nmol V cm^{-2} yr^{-1}) also matches the accumulation rate of vanadium in the sediments. This emphasizes the refractory

Table 1 Hydrographic data and vanadium concentrations in vertical profiles from the North Pacific Ocean

Depth (m)	Temperature (°C)	Potential salinity	O_2 (μmol l^{-1})	PO_4 (μmol l^{-1})	V (nmol l^{-1})
MANOP S					
1	26.97	34.676	205	0.08	32.0
50	26.86	34.671	229	0.03	32.6
100	13.47	34.588	12.5	2.21	33.3
150	11.51	34.730	14.3	2.27	34.4
200	10.87	34.720	15.2	2.30	34.9
300	9.936	34.693	24.0	2.39	35.0
350	9.61	34.679	11.6	2.40	35.3
400	9.321	34.664	12.5	2.50	35.3
450	8.97	34.643	7.1	2.61	35.0
497	8.435	34.609	10.0	2.75	34.8
546	7.868	34.575	7.7	2.75	37.1
595	7.47	34.561	7.0	2.97	36.5
693	6.34	34.526	13.2	3.03	36.1
742	5.901	34.527	14.4	2.96	37.4
890	4.985	34.533	29.9	3.00	36.6
988	4.518	34.555	40.0	3.08	36.5
1,185	3.853	34.569	48.4	2.95	36.0
1,280	3.592	34.578	68.0	2.72	35.6
1,478	3.122	34.596	65.3	2.73	36.5
1,675	2.722	34.611	76.3	2.66	34.9
1,872	2.362	34.628	88.2	2.54	36.4
2,070	2.11	34.642	97.2	2.35	35.7
2,267	1.96	34.657	108	2.38	35.9
2,677	1.794	34.666	117	2.37	36.2
2,975	1.698	34.673	127	2.34	36.3
3,472	1.551	34.681	138	2.25	35.3
4,625	1.444	34.693	167	2.14	36.1
4,772	1.442	34.695	175	2.16	36.4
4,904	1.432	34.694	169	2.18	36.6
Galapagos					
10	24.94		218	0.51	33.6
40	20.67		165	0.98	34.0
80	17.87		135		34.7
120			98	1.57	35.3
200	14.52		96	1.64	35.3
250	13.54		74	1.87	35.9
300	12.16		19	2.38	35.9
350	10.66		10	2.60	36.1
400	9.40		11	2.79	35.7
500	7.73		23	2.96	36.6
600	6.96		37	3.00	37.4
800	5.62		54		36.3
1,000	4.63	34.577	71	2.99	35.2
1,150	4.02	34.589	75		35.3
1,900	2.39	34.650	112	2.85	36.5
2,200	2.11	34.656	122	2.77	36.5
2,300	2.12	34.655	115	2.78	35.3
2,400	2.07	34.655	120	2.77	35.3
2,460	2.07	34.669	117	2.78	36.4
Subarctic Pacific					
30	8.24	32.499	305	1.10	29.8
100	4.86	32.893	275	1.54	29.5
200	3.93	33.758	130	1.99	34.4
400	3.79	34.009	60	2.74	33.3
500	3.68	34.126	47	2.58	34.3
600	3.48	34.190	36	2.58	32.8
800	3.13	34.284	29	2.88	34.2
1,000	2.79	34.347	23	2.98	33.8
1,250	2.47	34.419	26	2.93	34.7
1,500	2.21	34.462	36	2.64	34.7
2,000	1.84	34.526	60	2.77	34.2

Samples from MANOP, Site S (11°N, 140°W) were collected in May 1979. Galapagos samples (0°44′ N, 86°10′ W) were collected in March 1979, and those from the subarctic Pacific (50° N, 150° W) in June 1981.

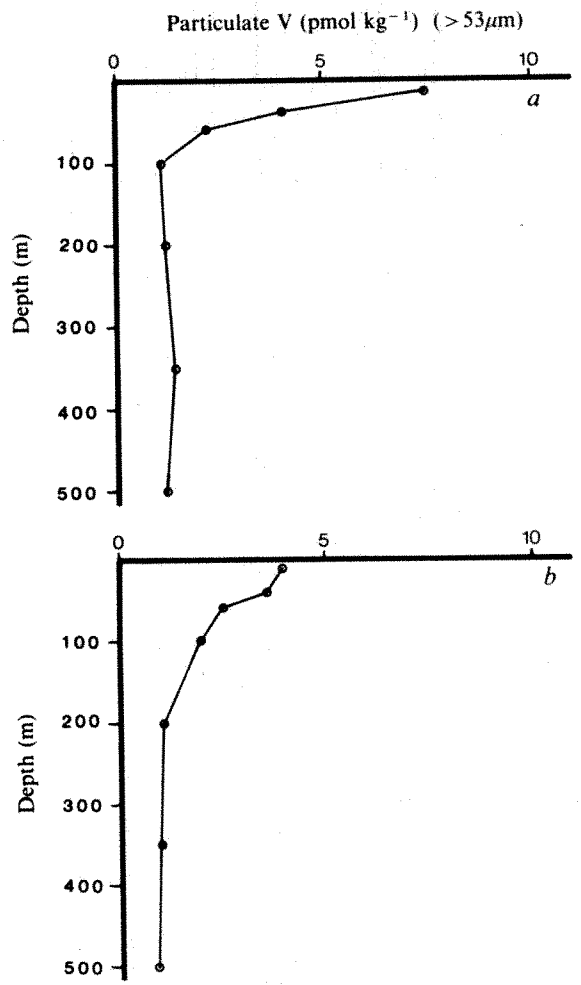


Fig. 1 Concentration of vanadium (pmol V per kg seawater) in particles larger than 53 μm. The samples were collected *in situ* by a filtration technique[2] that separates three particle size fractions (<1, 1–53, and >53 μm) from large volumes of water (2–20 m^3). Thus, the sampling method permits the collection of larger sized particles which, although rare, settle rapidly enough to dominate the vertical mass flux in most environments[1–3,25]. The size–frequency distribution of the particulate matter on the filters is determined microscopically and, when combined with estimates of settling rates for each particle class, yields an estimate of the total vertical mass flux. This is combined with the bulk chemical composition of the particulate material to estimate the flux of each chemical component. Particulate vanadium was determined by instrumental neutron activation analysis[26] of the >53 μm size fraction. Data shown are samples from the Galapagos site collected in: *a*, July 1976; *b*, March 1979.

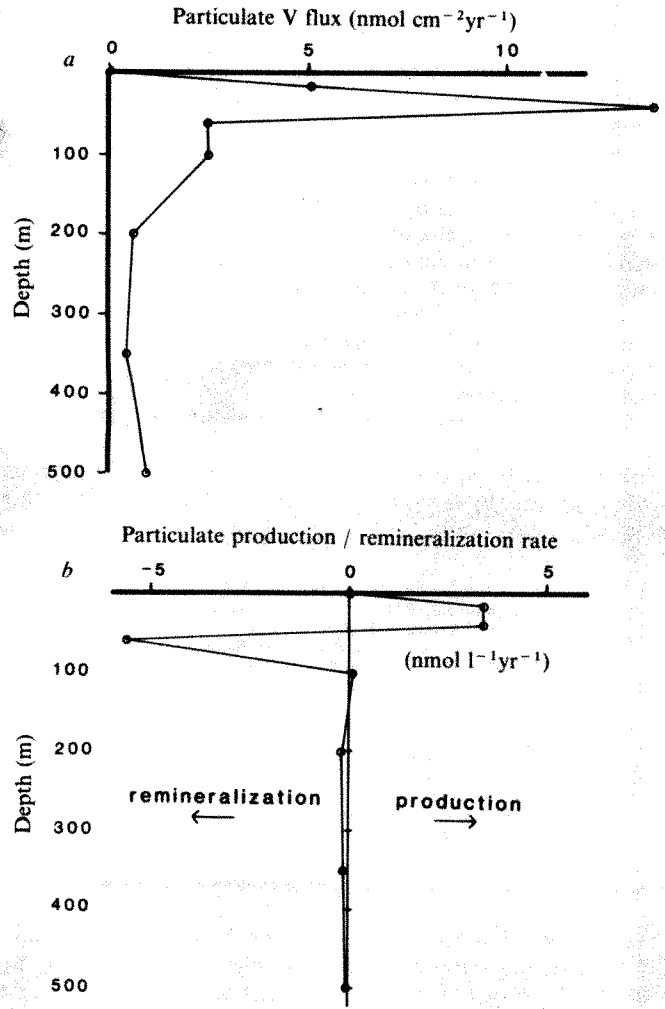


Fig. 2 *a*, Calculated vertical flux of particulate vanadium; *b*, resulting production or remineralization of vanadium due to changes in the particle flux.

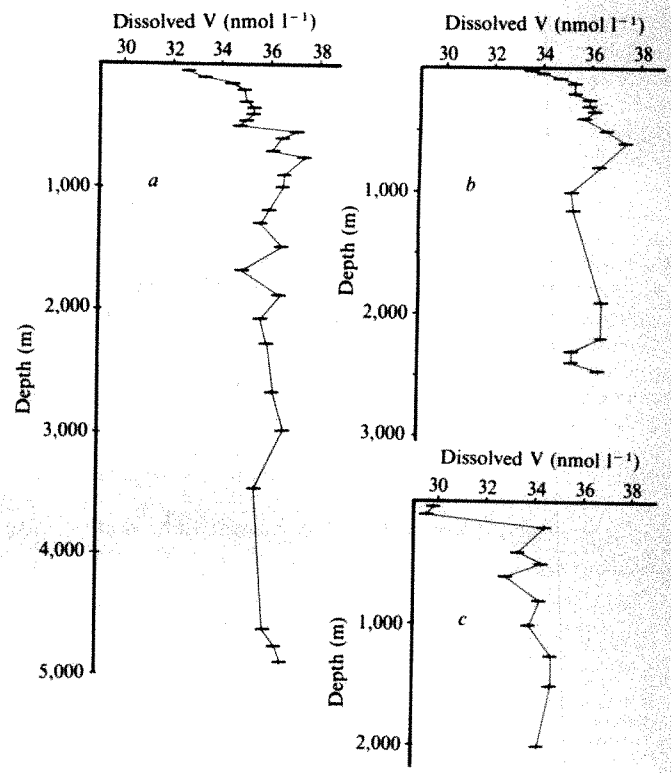


Fig. 3 Dissolved distributions of vanadium showing surface depletions: *a*, MANOP site S; *b*, Galapagos site; *c*, subarctic Pacific. The analysis for dissolved vanadium was made by atomic absorption spectrometry using a graphite furnace atomizer. For preconcentration of the vanadium, the acidified samples were buffered to *p*H 4 and the trace elements were co-precipitated with cobalt–APDC[16]. The method recovers both V(IV) and V(V) oxy-anions from seawater with an efficiency of 80%. The limit of detection using 35 ml of seawater is 1 nmol V per kg seawater; the precision is 0.4 nM (1σ) at 35 nM vanadium.

nature of the vanadium in these deep samples. Clearly, the 'biogenic' vanadium seen near the surface in the 1976 samples is very labile and does not contribute significantly to the deep flux.

This region of the oceans is subject to large seasonal as well as inter-annual variations in productivity associated with the complex equatorial current systems[6]. The 1976 data were collected during a low productivity event caused by El Niño conditions[3] which may have resulted in the observed differences between the two particulate vanadium profiles. The nature of the vanadium association with organic particulate matter is unknown, as is the case with most other trace metals[7]. Although there is a vanadium(III) oxygen carrying protein in some ascidians[8], its significance to pelagic vanadium cycling is doubtful. There may, however, by labile vanadium enrichments in related organisms such as salps. Other organovanadium complexes (such as tetrapyrroles) found in marine sediments[9] are stable diagenetic products of prophyrins.

Vertical flux estimates for vanadium from the 1976 profile are shown in Fig. 2*a*. These fluxes are based on the concentration data (Fig. 1*a*) and estimates of the bulk mass flux[3]. The flux reaches more than 10 nmol V cm^{-2} yr^{-1} at 60 m but drops off by an order of magnitude below 200 m depth. Based on the observed particle compositions[3], vanadium behaviour in the upper water column parallels the formation and decomposition of particulate phosphorus at a constant V/P ratio of 4×10^{-3}. Using this ratio with an estimate of the global flux of particulate phosphorus[10] (0.87 μmol P cm^{-2} yr^{-1}) yields an estimate of the flux of vanadium out of the surface ocean of 3.5 nmol V cm^{-2} yr^{-1}.

Assuming that the vertical profile is in steady state, a change in the flux as a function of depth requires *in situ* production or remineralization of particulate vanadium (Fig. 2*b*). If this change occurs solely through exchange with dissolved vanadium, the estimated fluxes also represent the dissolved depletion or regeneration rate. This rate can be compared with other fluxes in the marine vanadium cycle by using a simple vertical two-box model[7,11]. Depletion of vanadium from surface waters must be balanced by primary inputs, including riverine and atmospheric inputs as well as physical exchange with deeper water. The riverine[12] and atmospheric[6,13] inputs probably supply $\leqq$0.4 nmol V cm^{-2} yr^{-1} such that at least 85% of the particulate flux estimated from the LVFS data must be supplied by exchange of depleted surface water with the deep ocean. At an average volume exchange rate of 3.5 m yr^{-1} distributed over the ocean surface, the surface water reservoir should be depleted in vanadium by about 8 nmol l^{-1} relative to the deep reservoir.

When these flux estimates were completed[14], very little data existed for the concentration of vanadium in seawater[15], and none had been generated using currently-accepted sampling methods. Especially lacking were any high-quality profiles from the Pacific. Therefore, vanadium was analysed in samples collected from the Pacific specifically for trace metal analysis. The hydrographic data and concentrations of vanadium are given in Table 1 and the distributions are presented in Fig. 3. A surface depletion of V is clearly demonstrated, although the magnitude is about one-half that predicted by the particulate data. The average 'specific' vanadium (normalized to a salinity of 35.0) was 32.7 nM in these Pacific surface waters and 36.4 nM in the deep Pacific. Based on the pelagic data, the residence time of dissolved vanadium in the ocean with respect to its river input[12] is $\sim 5 \times 10^4$ yr.

Significant processes affecting the concentration of trace metals at the ocean margins (such as increased scavenging, diffusion from sediments, or inputs from rivers) have been identified through the examination of surface water samples collected on transects moving away from coastal regions[16–18]. The specific vanadium concentrations in surface samples taken along a transect off California (at 39° 38′N) had an average value of 32.4 nM (similar to the pelagic values) and showed no systematic change near the coast.

The marine geochemical cycles of metals are often affected by their involvement in redox cycles[19,20]. Major concentration changes result from direct changes in solubility as a function of oxidation state and scavenging by fresh hydrous metal oxides produced near oxygen interfaces. The specific vanadium concentration within the anoxic zone of Saanich Inlet, British Columbia was ~26 nM which is somewhat lower than the pelagic value. However, there were no systematic changes in the concentration of vanadium with depth that related to other redox parameters.

These pelagic distributions are consistent with other recently reported determinations of dissolved vanadium[21–24], although a slight surface enrichment has been reported in the western North Atlantic[23]. In view of the variability of particulate vanadium seen at the Galapagos site (Fig. 1*a*, *b*), it is not surprising that some variations in its dissolved surface depletion exist. The simple box model applied to estimate the surface depletion is, at best, a crude approximation of the oceans and the 1976 data set used to derive the flux (Fig. 2) may not be representative of the mean biogenic flux of vanadium. Therefore, the factor of 2 agreement between the predicted and measured distributions is encouraging. There is certainly much more to learn about the detailed chemistry of vanadium and other metals in the oceans. This investigation demonstrates how both particulate matter and dissolved distributions can be coupled to study these biogeochemical cycles.

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Radium fluxes from a salt marsh

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Dissolved ^{226}Ra (half life 1,600 yr), ^{228}Ra (half life 5.7 yr) and ^{224}Ra (half life 3.64 days) activities in estuarine and coastal waters higher than values reported for open ocean or river waters have been explained by desorption and diffusion of radium from coastal and estuarine sediments[1–4]. Fluxes of radium isotopes from sediments in Long Island Sound, the Hudson River estuary, Chesapeake Bay and Winyah Bay, South Carolina, have been estimated from mass balances, diffusive bottom fluxes and desorption from suspended sediments[4–7]. These estimates may not be correct if salt marshes which are intimately associated with estuaries and the coastal ocean are an additional source or sink of radium[4]. In this study Bly Creek, a small marsh-tidal creek system near Georgetown, South Carolina, was chosen to determine the flux of ^{228}Ra and its great granddaughter, ^{224}Ra, from the sediments to the tidal creek water. We find that the highest activities of ^{224}Ra and ^{228}Ra in the tidal creek waters occur during low tide, but ^{226}Ra activity remains relatively constant throughout the tidal cycle. Fluxes of ^{224}Ra and ^{228}Ra calculated from mass balances required to support the observed activities in the creek waters and measured directly using a flux chamber are consistent. These measurements reveal a large input of radium from the marsh sediments to the overlying waters. To produce the necessary fluxes, irrigation and bioturbation must replace interstitial water in the top 5 cm of the marsh sediments every 2–3 days.

Surface water samples collected at approximately 40-min intervals over two tidal cycles were processed for radium isotope determinations[8]. Water velocity and depth were measured simultaneously. The activity ratios ^{228}Ra/^{226}Ra and ^{224}Ra/^{226}Ra were determined by γ-ray spectrometry and absolute ^{226}Ra activities were determined by radon emanation[3,4]. Absolute ^{224}Ra and ^{228}Ra activities were calculated from the activity ratios and the absolute ^{226}Ra activities. ^{226}Ra activities remained constant and net water flux was essentially zero throughout the study.

The ^{224}Ra and ^{228}Ra data describe two straight lines when plotted against the height of water at the sampling site in Bly Creek (Figs 1, 2). The highest activities of radium occur during the lowest tide heights. We conclude that this linear relationship between water depth and radium activity must represent dilution of high-activity marsh waters with low-activity ocean water. Thus, radium must be supplied to the creek waters from the marsh sediments to maintain the high activities observed at low tide. The only other potential source, groundwater, can be excluded because salinities did not vary significantly in Bly Creek during the study.

If Ra in the marsh system is in steady state, that is, if the Ra exported during ebb tide is balanced by production and release of Ra from marsh sediments, we can use mass balances to determine Ra fluxes from the sediments.

$$F_{Ra} = \frac{V_{out}Ra_{out} - V_{in}Ra_{in}}{A\Delta t} \quad (1)$$

where F_{Ra} is the flux of Ra from the sediment; V_{out} is equal to V_{in}, the volume of water exchanged during one tidal cycle; Ra_{out} is the Ra exported from the marsh (d.p.m. l^{-1}); Ra_{in} is the Ra imported from the coastal ocean (d.p.m. l^{-1}); A, is the area of marsh flooded at high tide; and Δt, is the time in one tidal cycle (12 h).

Measurements of Ra isotopes in nearby coastal waters reveal that activities of ^{224}Ra and ^{228}Ra are quite low (of the order of 0.2–0.3 d.p.m. l^{-1})[8,9] and for a first approximation can be neglected when compared with the activities observed in the marsh waters (1–3 d.p.m. l^{-1}). Thus, equation (1) simplifies to

$$F_{Ra} = \frac{V_{out}Ra_{out}}{A\Delta t}$$

Topographic data are used to estimate the volume of water

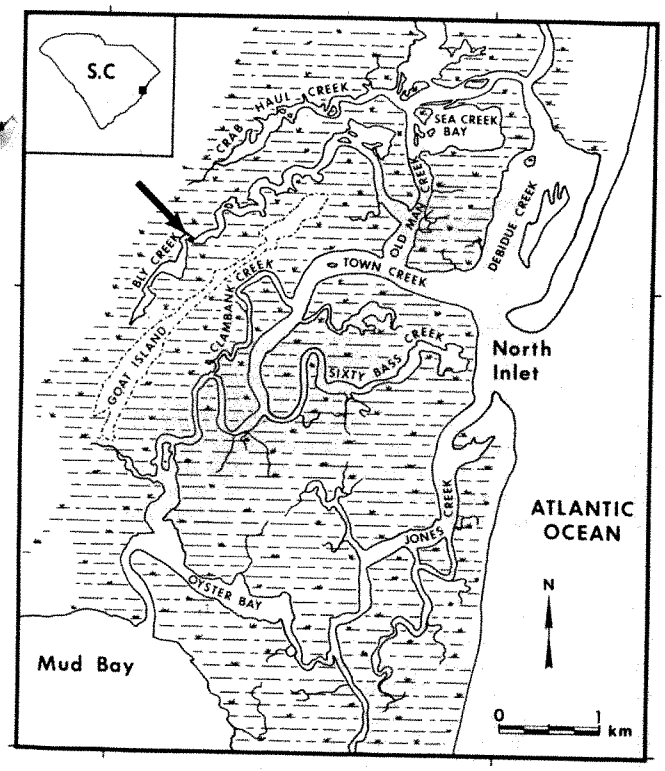


Fig. 1 Map of marsh surrounding North Inlet, South Carolina. Location of primary sampling site on Bly Creek (33°20′ N 79°12′ E) is indicated by the arrow. Bly Creek drains the sandy sediments between ancient beach ridges, one of which is labelled Goat Island.

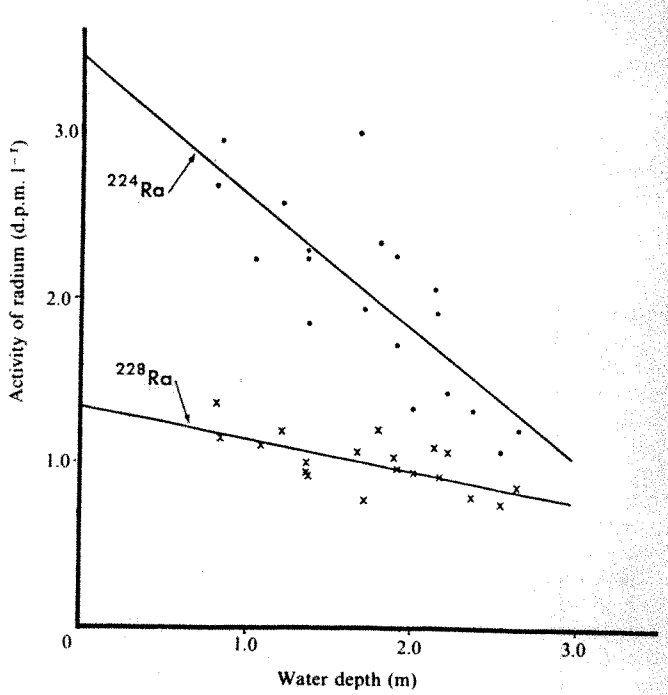


Fig. 2 The activity of radium versus the water depth in Bly Creek on 15 and 16 October 1982. Correlation coefficients for ^{224}Ra and ^{228}Ra are −0.795 and −0.667, respectively.

leaving the marsh on the ebb tide, V_{out}. The average activity of radium over one tidal cycle, Ra_{out}, is 2.1 d.p.m. l^{-1} for ^{224}Ra and 1.0 d.p.m. l^{-1} for ^{228}Ra (Fig. 2). Thus, the fluxes of ^{224}Ra and ^{228}Ra are 2×10^{-7} d.p.m. $cm^{-2}\,s^{-1}$ and 1×10^{-7} d.p.m. $cm^{-2}\,s^{-1}$, respectively.

To determine whether these fluxes are realistic, we devised a flux chamber consisting of a 25-cm diameter, 1.4-m long polyvinylchloride pipe closed at one end except for an air vent. The open end of the chamber is inserted about 15 cm into the sediment. An opening just above the sediment is connected by flexible hose to a conveniently located column which is secured near the sediment surface and filled with acrylic fibre impregnated with manganese dioxide (Mn-fibre)[10]. The apparatus is installed at low tide in an area free of surface water; as the tide rises, water passes through the Mn-fibre column to fill the chamber. The Mn-fibre strips all radium isotopes from the incoming water. At high slack tide the Mn-fibre column is replaced with a fresh Mn-fibre column. As the tide falls, any radium which may have moved from the sediment into the chamber is collected by the Mn-fibre column as water drains from the chamber. Fluxes of ^{224}Ra and ^{228}Ra obtained by this method are 6×10^{-7} d.p.m. $cm^{-2}\,s^{-1}$ and 2×10^{-7} d.p.m. $cm^{-2}\,s^{-1}$, respectively.

The fluxes calculated from the tidal cycle data agree with the fluxes measured by the chambers to within a factor of 3. Considering the assumptions used in the tidal cycle calculation and possible variation in fluxes throughout the marsh, this agreement is very good.

Decay of ^{228}Th (half life 1.9 yr) produces ^{224}Ra in the marsh sediments. Using 0.5 d.p.m. ^{228}Th cm^{-3} of sediment (based on initial analyses) gives a production term of 5.9×10^{-6} d.p.m. ^{224}Ra $cm^{-2}\,s^{-1}$ over a depth of 5 cm. We chose 5 cm as the depth from which ^{224}Ra can be derived because: (1) this is approximately the top of the dense root zone of the *Spartina*, and (2) preliminary measurements show ^{224}Ra in interstitial waters to be depleted in the top 5 cm but constant below 5 cm. Comparison of this potential source with the flux necessary to support the ^{224}Ra in the water column (from the more conservative tidal cycle data) reveals that only about one-third of the ^{224}Ra produced in the top 5 cm of sediment need be mobilized to yield the required fluxes. A similar calculation for ^{228}Ra reveals that to support the observed flux, ^{228}Ra must be removed from at least 25 cm of sediment if this is a steady state flux. However, the ^{228}Ra flux may be periodic with seasonal gains and losses of the longer-lived Ra isotopes in the sediments.

Three mechanisms may be driving this exchange process: (1) molecular diffusion, (2) advection of interstitial water from the sediments due to turbulence in the overlying water and tidal pumping, and (3) turbation and irrigation by organisms. To estimate the scale length (L) over which molecular diffusion is effective we use the relationship $L=\sqrt{D/\lambda}$ where D is the effective diffusion coefficient of Ra, 3.4×10^{-8} $cm^{-2}\,s^{-1}$ (assuming a temperature of 18 °C, a tortuosity of 2.0, and an adsorption coefficient of 100)[11–13], and λ is the decay constant of ^{224}Ra, 2.2×10^{-6} s^{-1}. Thus, the path length is 0.12 cm. Our preliminary interstitial measurements indicate that ^{224}Ra is removed from at least 40 times this depth; therefore, other factors must be increasing the exchange. Because the flux chambers show Ra fluxes almost as high as the tidal cycle data require and as the closed chambers inhibit physical turbulence, mixing due to turbulence of interstitial water is probably not the major transport mechanism of radium from the marsh sediments. In addition, a preliminary experiment indicates that a significant amount of water is not entering the chamber due to tidal pumping.

However, bioturbation and irrigation may indeed increase the flux of radium from the sediments by increasing the effective surface area of the marsh and by physically moving water of higher radium activity to the overlying water. Fiddler crab burrows dot the sediment surface from the creek banks to the low *Spartina alterniflora*, *Salicornia* areas of the marsh and effectively increase the sediment surface area. Worms, fiddler crabs and other residents of the marsh sediments also rework the sediments, physically moving water containing high radium activity towards the surface of the marsh.

The activities of ^{224}Ra in the water at low tide (C_L = 3 d.p.m. l^{-1}) and high tide (C_H = 1 d.p.m. l^{-1}) and in the pore water at 5 cm depth (C_P = 12 d.p.m. l^{-1}) as well as the volume of water which flushes the marsh during one tidal cycle

$V_{in} = V_{out}$) constrain the volume of water which is mixed through the upper 5 cm of the sediment (V_{mix}) as follows:

$$V_{out}C_L = V_{in}C_H + V_{mix}C_P - V_{mix}C_H$$

Using the observed data $V_{mix} = 9 \times 10^9$ cm^3. Thus, the residence time of water in the upper 5 cm of sediment can be determined from the following equation

$$\tau = \frac{\text{porosity} \times \text{depth} \times \text{area}}{V_{mix}/12\text{ h}}$$

and is of the order of 2–3 days.

Our study indicates that salt marshes are important sources of radium isotopes to coastal waters. The short residence time of water in the top centimetres of the salt marsh sediments and the high flux of radium out of the sediments demonstrate that marshes are extremely active chemical systems able to respond to changing conditions on a time scale of days. These observations have important implications for our understanding of the cycling of nutrients, metals, carbon and pollutants in coastal systems.

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Ultraviolet sensitivity in hyperpolarizing photoreceptors of the giant clam *Tridacna*

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The giant clam *Tridacna* is a common shallow-water inhabitant of Indo-Pacific coral reefs[1]. These animals contain large populations of photosynthetic zooxanthellae in hypertrophied siphonal lobes which extend well beyond the edge of the shell for maximum exposure to light[2–4] (Fig. 1). Protection for these large fleshy lobes is visually mediated and consists of a partial siphon retraction and valve adduction, a behaviour which gives rise to a forceful, startling jet of seawater[5]. Numerous eyes (up to several thousand in large animals[6]) located along the margin of the siphon comprise a visual system which presumably mediates the defensive withdrawal reflex. Here we present the first report on the physiological properties of the visual system in *Tridacna*. Intracellular electrophysiological recordings have revealed two distinct types of retinal cells: both are hyperpolarized by light but only one generates axonal spikes. We also describe cells with differential colour sensitivity, including receptors that are sensitive to ultraviolet (UV) light. Ultraviolet sensitivity, although not previously reported in molluscs[7], may be an adaptation in *Tridacna* to the environmental requirements of a host for algal symbionts.

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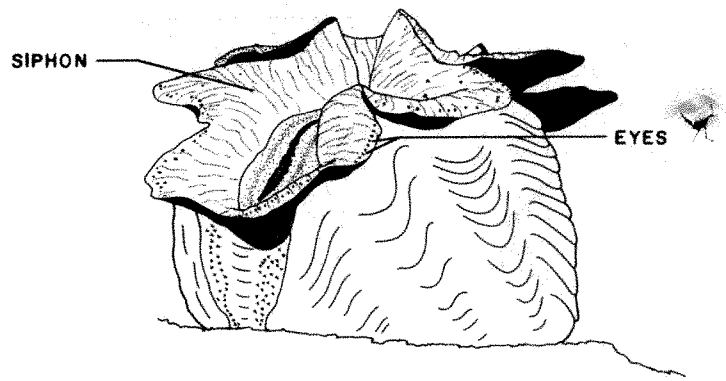


Fig. 1 *Tridacna maxima* L., drawn from a 35 mm colour slide projection (see cover) showing the posterior lobes of the siphon, the slit-like inhalant aperture and the right valve. The siphon lobes are fully extended, as they are in bright sunlight, with the eyes (bold dots) forming a line around the lateral margins. Shell length, 16.5 cm.

Tridacna were collected at Davies Reef in northern Queensland. The eyes are visible at the mantle surface as clear lens-like structures surrounded by a dense accumulation of pigmented zooxanthellae. Retinal cells line the back of the eye capsule and an optic nerve extends into the adjacent tissues[3,8]. In these experiments the epithelium was removed and the lens gently expressed to expose the retina. Glass micropipettes containing 3 M KCl were then lowered directly onto the retina for intracellular recording. Light from a xenon arc source was shone on the eye using a fibre optic light guide and was controlled by an electronic shutter in combination with neutral density and interference filters.

In recordings from over 100 light-sensitive cells, the receptor potentials were exclusively hyperpolarizing (Fig. 2). However, two distinct classes of cells exist: spiking (S) and non-spiking (NS). Of the cells encountered in these experiments, a slight majority were NS (52%), although both types were observed in most eyes. That the absence of spikes in NS cells is a physiological characteristic of these receptors, and not an artefact due to injury from electrode penetration, was established by the following observations: (1) injury discharges, which were occasionally observed in S cells, were never seen in NS cells; (2) S-cell spikes remain for the duration of stable penetrations in most instances, occasionally lasting for 30–60 min; and (3) additional physiological characteristics invariantly correspond with the presence or absence of spikes. For example, NS cells have low resting membrane potentials in the dark (-14.4 ± 6.7 mV, ±s.d.) and large receptor potential amplitudes (Fig. 2*a*); responses greater than 100 mV have been observed in fully dark-adapted preparations, but these potentials are readily attenuated by successive light stimuli and adapt to an intermediate level of polarization (-37.8 ± 12.7 mV) under steady illumination at high intensities (Fig. 2*b*). In contrast, S cells have a larger resting membrane potential (-43.8 ± 6.6 mV) than NS cells, but the receptor potential amplitude is smaller (14.0 ± 5.2 mV) and not affected by light or dark adaptation. The difference in adaptation sensitivity is most striking in response to light flashes of long duration where, by comparison with NS cells, the S cell maintains a steady level of membrane hyperpolarization; in some instances there is a subsequent gradual depolarization by a few millivolts (for example, 3–4 mV in Fig. 2*c*). However, there was very little difference in response latency between S and NS cells.

As with the cell represented in Fig. 2*c*, many S cells are spontaneously active in the dark and the spikes, 3–8 mV in amplitude, are inhibited by light. In response to dimming, bursts of spikes (60–80 Hz; 0.5–2.0 s) are generated on the depolarizing phase of the receptor potential, with the frequency and duration of the burst being increased by longer periods of light exposure. Thus, S cells are sensitive to light adaptation, although not directly in terms of the receptor potential waveform.

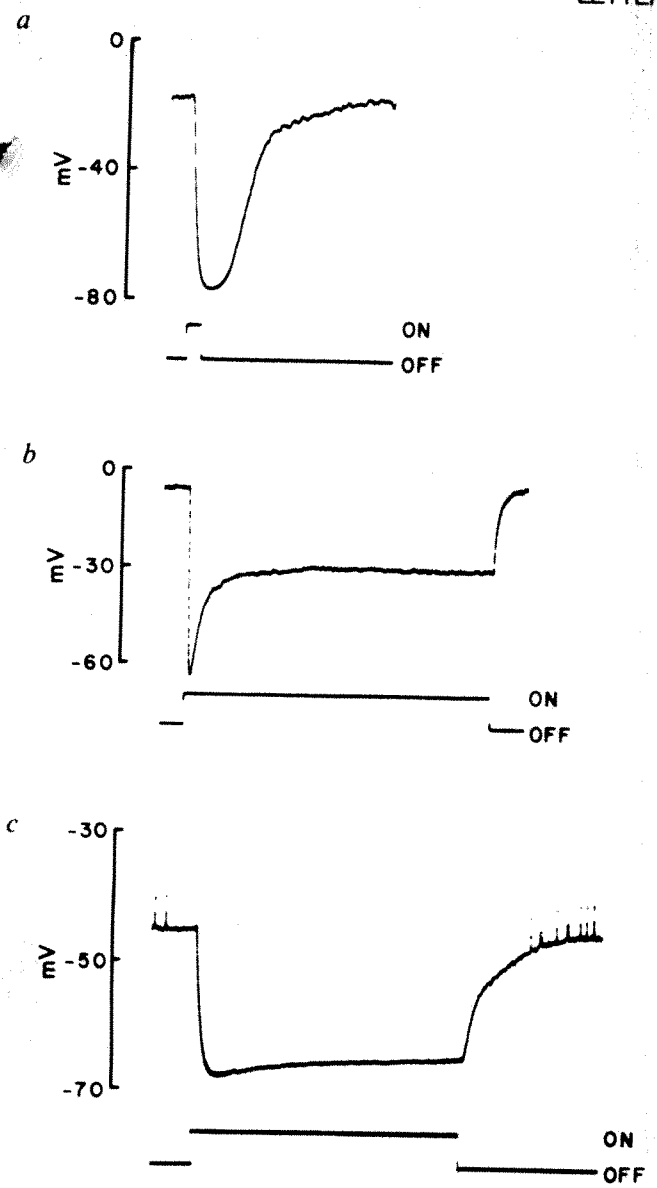


Fig. 2 Hyperpolarizing receptor potentials recorded intracellularly from *Tridacna* retinal cells. Receptor potentials from non-spiking (NS) photoreceptors in response to *a*, a brief light flash (250 ms); *b*, a light-adapting stimulus (2 min). NS receptors have a low resting potential in the dark and adapt rapidly to light. *c*, Receptor potential from a spiking (S) photoreceptor in response to an extended period of light stimulation (1.5 min). S receptors have higher resting potentials than NS cells but the light-mediated hyperpolarization does not adapt to light. Spikes occur spontaneously and in bursts on the depolarizing phase at light-off.

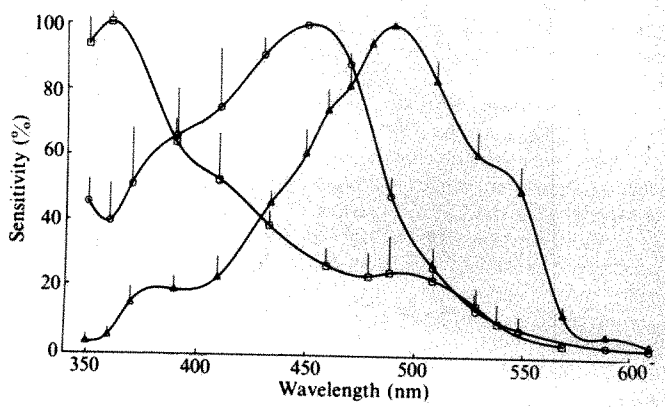


Fig. 3 Spectral sensitivity of *Tridacna* photoreceptors. Relative sensitivity, as measured by the amplitude of the hyperpolarizing receptor potential, plotted against wavelength. Individual photoreceptor sensitivity curves fall into three groups and the results shown are the means (±1 s.e.) for cells in each category with curve-smoothing based on a spline function: $\lambda_{max} = 490$ nm, $n = 7$ (Δ); $\lambda_{max} = 540$ nm, $n = 2$ (○); $\lambda_{max} = 360$ nm, $n = 4$ (□). Constant intensity light flashes, of 100–200 ms duration in different experiments, were given at 15-s intervals—stimulus parameters determined not to be light adapting.

To test the colour sensitivity of *Tridacna* photoreceptors, receptor potential amplitudes were measured in response to monochromatic light flashes from 350 to 610 nm. Data were used only from experiments in which stable responses were obtained for V–log I test sequences preceding and following the spectral test series. Surprisingly, we found individual receptors with sensitivity peaks at three specific wavelengths (Fig. 3): of 13 cells meeting the criteria, seven were green-sensitive ($\lambda_{max} = 490$ nm), two were blue-sensitive ($\lambda_{max} = 450$ nm), and four were sensitive to UV light ($\lambda_{max} = 360$ nm). All of the NS cells in this group were sensitive to green light, whereas S cells were represented in each category including one green- and all of the blue- and UV-sensitive photoreceptors. Behavioural sensitivity to various colours of light was also tested. Under a low level of white background illumination, siphon retractions were recorded in response to 3-s interruptions of adapting light at selected wavelengths. The siphon responded to conditioning light over the range of colours used in the physiological studies, but the threshold intensity for UV light (350 nm) was approximately four times greater than that for green light (490 nm).

Tridacna photoreceptors exhibit several interesting physiological features. In contrast to the scallop whose eyes contain both depolarizing (proximal) and hyperpolarizing (distal) photoreceptors[9], each directly initiating axon spikes, *Tridacna* has two types of hyperpolarizing receptor but only one has the ability to generate spikes. It is possible that *Tridacna* NS cells may not be photoreceptors, but instead are glial cells passively responding to adjacent receptors. This explanation is unlikely as (1) other cells (glial candidates) with no response of any kind are frequently penetrated, (2) response latencies are equivalent for both receptor cells, and (3) Lucifer yellow injections of both S and NS cells show axons projecting from the cell soma (unpublished results). Non-spiking photoreceptors, in addition to the vertebrate rods and cones, are now well known in arthropods (for example, barnacles[10]) but they have not previously been reported in molluscs and their physiological role is unknown. The S cell, on the other hand, is the physiological equivalent of the scallop distal photoreceptor: common features include a hyperpolarizing receptor potential which does not adapt to light[9], and an 'off-response' burst of spikes, the intensity of which is directly related to preceding light conditions[11]. Spike output therefore appears to correspond to an accumulating excitatory component of the light response which is masked by a separate 'primary' light-inhibiting effect expressed by the hyperpolarizing receptor potential. It has been suggested[12] that primary light inhibition may be characteristic of bivalve visual systems where the predominant light response in a variety of pallial photoreceptor systems is to dimming, that is, to a shadow[12–14], and where behaviourally relevant shadow reflexes are the norm[12,15].

Of even greater interest is the existence of receptors sensitive to short wavelengths of light, specifically to UV. In previous studies encompassing each of the three major molluscan classes[7,12,13], the maximum sensitivity for rhodopsin-like photopigments was typically around 490 nm, a value corresponding with that of the NS cells in *Tridacna*. UV light receptors represent a significant departure from this pattern, and it is doubtful that the electroretinograph or compound nerve-recording techniques used in several of the previous studies would have obscured the presence of UV receptors. *Tridacna* is a unique mollusc in several

respects, and recognition of these features can assist in the formulation of hypotheses to account for UV sensitivity in this genus.

Of the previous molluscs tested, including several intertidal bivalves, all are able to limit their exposure to UV light by means of protective shells or cryptic locomotor behaviour, and/or they occupy temperate neritic waters where this spectral component is rapidly absorbed at the surface. In contrast, *Tridacna* occurs in a shallow-water tropical habitat, including reef flats that are partially exposed at low tide, where turbidity is low and UV penetration maximal[16,17]. In these regions UV irradiation is known to be a major limiting factor in the distribution of marine invertebrates[18]. Despite this fact, *Tridacna* intentionally exposes its siphonal lobes to intense light and furthermore does not normally withdraw completely into its shell unless provoked repeatedly. UV light receptors may somehow be involved in this positive siphonal phototropism, which in turn may be correlated with the fact that the algal symbionts use a broad spectrum of light (300–700 nm) for photosynthesis[19]. Alternatively, the UV receptors may be functional components of the defensive withdrawal reflex, a behavioural possibility established in the present experiments and further supported by the fact that UV sensitivity is represented in the class of receptors that are physiologically adapted to respond directly to shadows. Whatever the precise role, it seems that *Tridacna* has adapted a range of spectral sensitivities within its population of retinal cells to match the colours present in its environment.

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Threshold channels—a novel type of sodium channel in squid giant axon

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Sodium channels in nerve and muscle cells are functionally similar across wide phylogenetic boundaries[1] and are usually thought to represent a single, homogeneous population that initiates the action potential at threshold and unerringly transmits it along the surface membrane. In marked contrast, many cell types are known to have several distinct potassium permeability systems[2,3]. Distinguishable populations of Na channels have been reported in a few cell types, however, including denervated skeletal muscle[4], embryonic cardiac muscle[5], Purkinje cell somata[6] and non-myelinated axons at low temperature[7]. We report here that in squid giant axon, in standard experimental conditions, there are two functionally distinct populations of Na channels. The newly discovered population accounts for only a few per cent of the total Na permeability. The channels are selectively activated by small depolarizations and have very slow closing kinetics. Because these channels activate at voltages near the resting potential and tend to stay open for long times, they must dominate behaviour of the axon membrane in the threshold region for action potential initiation.

Experiments on *Loligo pealei* were performed at the Marine Biological Laboratory, Woods Hole, Massachusetts. Cleaned axons were internally perfused and voltage-clamped as described previously[8,9]. Improvements in the electronic apparatus were important to this study in allowing the resolution of very small ionic currents ($<1\ \mu A\ cm^{-2}$). For measuring Na current (I_{Na}) plus gating current (I_g), the external solution contained (in mM): 60 (or 120) NaCl, 50 $CaCl_2$ and 352 (or 452) Tris *p*H 7.0 (Sigma) (60 or 120 Na; see figure legends). Gating currents were recorded by replacing all Na with Tris *p*H 7.0 and adding 200 nM tetrodotoxin (TTX). I_{Na} records were subsequently generated by subtraction. The internal solution was K-free; both tetramethylammonium (TMA) and Cs were used as K substitutes with similar results (150 mM as glutamate salt and 50 mM as fluoride salt). Internal solutions also contained (in mM): 10 Tris *p*H 7.0 and 560 sucrose. Pronase (Sigma type XIV) was internally applied (0.5 mg ml^{-1}) in some experiments to destroy inactivation as indicated below. Temperature was 8 °C, holding potential −80 mV, and we compensated for 2–3 $\Omega\ cm^2$ of series resistance. Particular care was taken to test for contamination of I_{Na} by end effects; tests included applying TTX to the axon ends in the air gaps, reducing the external Na concentration to as low as 30 mM, and comparing currents measured from different regions of the same axon. The phenomena described below were not affected by these control procedures.

The new channels that are activated in the threshold region were first recognized by their unusually slow closing kinetics. In Fig. 1*a*, the trace obtained in 120 mM Na shows the activation of $I_{Na} + I_g$ during a pulse to −50 mV as Na channels open, and the decay of current when the voltage is returned to −80 mV as the channels close. The tail current at −80 mV clearly has a fast and a slow phase of decay. The same procedure in the absence of external Na (0 Na, no TTX) shows an outward I_g transient at the beginning of the pulse which has the same initial amplitude as in 120 mM Na. After the return to −80 mV, I_g is inward and fast. The slow component is not seen in 0 Na and thus can be identified as Na current. Other experiments also showed it to be TTX-sensitive. Subtraction of the traces in Fig. 1*a* yields I_{Na} (Fig. 1*b*), and two components of tail current differing in decay rate by roughly an order of magnitude are still evident. Similar results were seen in every axon (total of 17) in which this phenomenon was examined over a 3-yr period.

Fast and slow components of I_{Na} decay accompany a variety of activating pulse amplitudes and durations. Following large pulses, however, the slow component constitutes a tiny fraction of the total current, and perhaps for this reason has been previously overlooked. Figure 2*a* illustrates the decay of I_{Na} in a Pronase-treated axon after a small pulse (−40 mV, 15 ms) and after a large pulse activating nearly all of the Na conductance (+40 mV, 3 ms). The two traces have very different initial ampli-

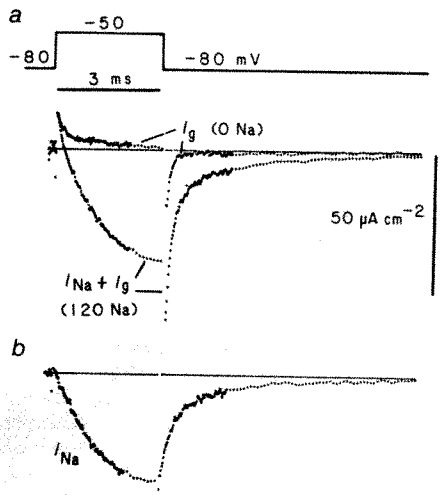


Fig. 1 Time course of Na current (I_{Na}) and gating current (I_g) activation at −50 mV, and deactivation at −80 mV. *a*, $I_{Na} + I_g$ (120 mM external Na) accompanying a 3-ms pulse is compared with I_g alone (0 Na). *b*, I_{Na} generated by subtraction of the traces in *a*. I_{Na} has clear fast and slow components of deactivation. JN243G; 120 mM Na, 200 mM TMA; Pronase-treated.

tudes, but they converge between 1.5 and 2 ms and decay slowly thereafter. Ignoring the small slow component, the +40 mV tail can be fitted well by a single exponential with time constant 150 μs. The −40 mV trace can be fitted with the sum of two exponentials having time constants of 180 μs and 1.9 ms—this disparity in values indicates distinct fast and slow phases of I_{Na} decay.

Figure 2*b* shows tail currents for a series of activating voltages. Traces for the three largest pulses (−45, −40 and +40 mV) have been truncated to allow the slow tail current to be plotted at high gain. Durations of the pulses ensured that the amplitude of the slow component was maximal in each case. The slow component increases with activating voltage up to ~−40 mV, where it reaches its maximum size. Its amplitude after the +40 mV step is the same. Activation kinetics of the channels underlying the slow tails are voltage-dependent and become faster at more positive voltages. Thus, 3 ms at +40 mV is as effective as 10–15 ms at −40 mV.

Each trace in Fig. 2*b* has both a fast and slow decay phase, but the two components differ greatly in relative magnitude, depending on the activating voltage. For the −55 mV pulse, the fast and slow components are roughly equal in size. Above −40 mV, only the fast component continues to grow, and it is much larger than the slow one after a step to +40 mV (Fig. 2*a*).

These results are very difficult to explain on the basis of a single, homogeneous population of Na channels having multiple conducting states. Instead, they support the existence of a small group of Na channels that are activated over a relatively negative voltage range and have very slow closing kinetics. The fast component of Na tail current reflects activation of a much larger group of 'normal' channels that have fast closing kinetics and activate at more positive voltages. Figure 3 shows the fraction of open channels plotted as a function of membrane potential for both populations. Near the resting potential the percentage of slowly closing channels activated (circles) is far larger than the activated fraction of normal channels. Small depolarizations to the region of action potential threshold (that is, −60 to −55 mV) would selectively activate the new channels. Although the total permeability contributed by this population is small (~3% of the total in Fig. 3), the channels can carry enough current to depolarize the membrane far beyond threshold for an action potential. Our results suggest that at least half of the channels that are open at threshold (for example, −55 mV in Fig. 2*b*) are of the new type. Thus, we label them 'threshold' channels, as they must dominate behaviour in the threshold region.

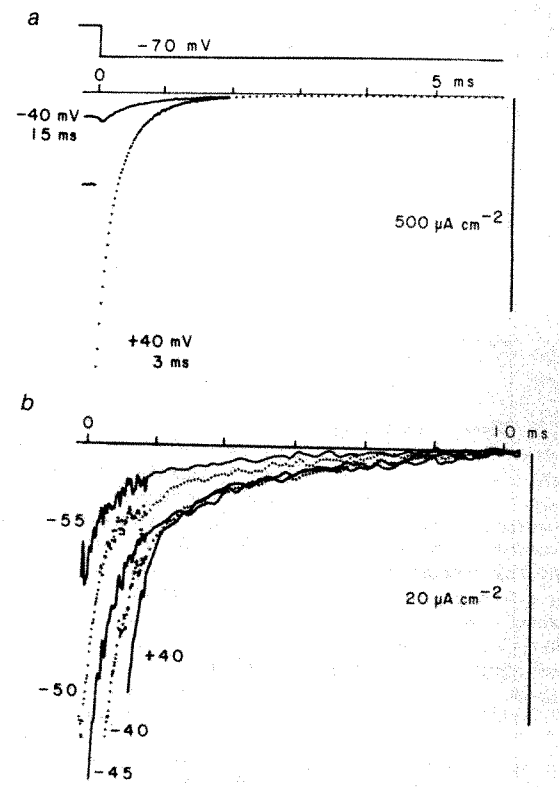


Fig. 2 Behaviour of fast and slow components of Na tail currents as a function of activating voltage. *a*, Currents recorded at −70 mV following a small (to −40 mV, for 15 ms) or large (+40 mV, 3 ms) depolarization. The axon was Pronase-treated. The initial amplitude of the tail currents is proportional to the number of channels opened. At −40 mV the number of open channels is ~10% of that at +40 mV. Although initial amplitudes are very different, the two traces converge between 1.5 and 2 ms. *b*, Tail currents for a series of activating voltages showing that the amplitude of the slow component saturates at ~−40 mV. Early portions of the traces for the three largest pulses are truncated; initial amplitudes were (in μA cm^{-2}) 40.5 (−45 mV), 69 (−40 mV) and 599 (+40 mV). Pulse durations were 15 ms, except for 3 ms at +40 mV. JN223H; 120 mM Na, 200 mM Cs.

The points for threshold channels in Fig. 3 can be adequately fitted by a two-state model having one open and one closed state. The solid curve assumes that the equilibrium constant for the transition from open to closed varies exponentially with voltage, changing e-fold in 4.5 mV. In terms of a Hodgkin–Huxley-like model[10] this corresponds to control of the channel by a single gating particle having 5.5 electronic charges associated with it. The normal channels require a multi-state model (several gating particles in Hodgkin–Huxley terms), making the conductance–voltage relation much less steep near its midpoint. The limiting steepness at very negative voltages may be similar to that for the threshold channels. Na conductance in Pronase-treated axons has been reported to change e-fold in 4.2 mV[11] to 5.3 mV[12]. These values may require reevaluation, however, because of the contribution made by threshold channels.

Sodium channels are normally inactivated during a maintained depolarization, and threshold channels share this property. Figure 4 shows three I_{Na} tails following the specified activating pulses from an axon with its inactivation mechanism intact, that is, not Pronase-treated. After 0.3 ms at +40 mV or 3 ms at −40 mV, inactivation has not progressed very far, and the slow components in those traces are identical. Both are about as large as can be recorded in a fibre with the inactivation mechanism functioning. Inactivation proceeds rapidly at +40 mV, however, and after 3 ms the slow component is virtually absent. Initial tail amplitude was also greatly reduced. Thus, both normal and threshold channels are inactivated with a rapid time course at +40 mV.

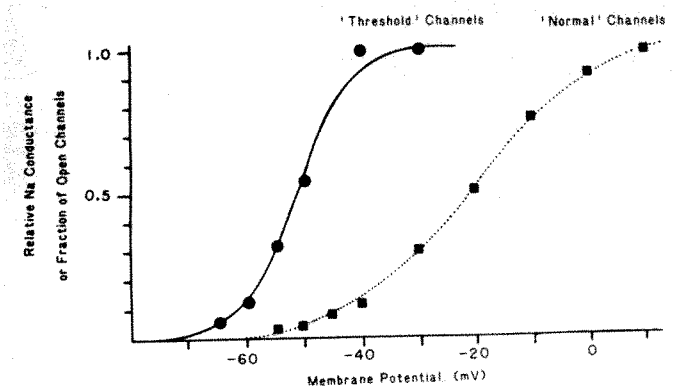


Fig. 3 Steady-state voltage dependence of Na conductance for 'threshold' versus 'normal' channels obtained in a Pronase-treated axon. Maximum conductance was 0.13 mS cm^{-2} for threshold channels as opposed to 4.3 mS cm^{-2} for normal channels, all measured in 60 mM Na. Conductance of normal channels (■) was measured at −80 mV as $\Delta I_{Na}/\Delta V$, 30 μs after termination of an activating depolarization to the indicated membrane potential[11]. The dotted curve was drawn by eye. Conductance of threshold channels (●) was estimated from tail current amplitude (at −80 mV) 2.5 ms after termination of the activating pulse. The solid curve represents a Boltzman distribution between open and closed channels such that the fraction of open channels = $e^{(V-V_{1/2})Z/25}/[1+e^{(V-V_{1/2})Z/25}]$ where V is membrane voltage (in mV), $V_{1/2} = -51$ mV, and $Z = 5.5$ electronic charges moving entirely across the membrane's electric field. Same axon as in Fig. 1; 60 mM Na, 200 mM TMA.

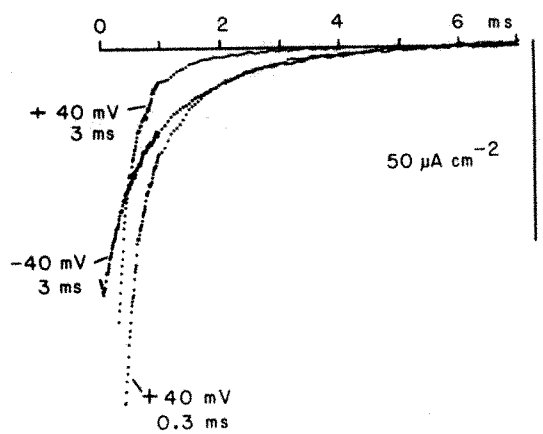


Fig. 4 Tail currents from a non-Pronase-treated axon with its inactivation mechanism intact. The slow component of current decay is identical following both the small pulse (−40 mV, 3 ms) and the brief, large pulse (+40 mV, 0.3 ms), neither of which produced significant inactivation. The slow component is nearly eliminated following the longer pulse to +40 mV (3 ms) which also produced substantial inactivation of the fast component. The +40 mV traces are truncated; initial amplitudes were 670 μA cm^{-2} (0.3 ms) and 320 μA cm^{-2} (3 ms). JN203G; 120 mM Na, 200 mM TMA.

Sodium channels similar to threshold channels have not been described for other preparations in studies using either conventional or single-channel techniques. Single-channel measurements should be a sensitive way of independently discerning these unusual channels. To keep the number of open Na channels conveniently small, these measurements are often made with small depolarizations, which would selectively activate threshold channels, at least in squid axon. The slow closing kinetics we report here as being diagnostic of these channels would be manifest in single-channel data as events with unusually long open times that were particularly evident between −70 and −40 mV. Unfortunately, single-channel data are not available for squid axon.

Thus, our results reveal the existence of a small but distinct second population of Na channels, the threshold channels. Compared with the normal channels, the threshold channels activate at relatively negative voltages and close very slowly once opened. Triggering the action potential is the probable function of the threshold channels. The normal Na channels, activating over a more positive voltage range, supply the large current required for rapid conduction. One advantage in this division of labour is noise reduction: there are fewer channels operating in the threshold region, and therefore less chance of a spurious action potential being triggered by the random, spontaneous opening of enough channels to reach threshold. The overall importance of threshold-type channels as described here is unknown. They must be important in squid axon for phenomena like repetitive firing. If similar channels exist in different regions of individual neurones, for example, dendrites or axon hillocks, they should have profound effects on determining the site of impulse initiation[6,13,14], and if they exist in autorhythmic cells, would greatly influence pacemaking or bursting behaviour.

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Current generated by backward-running electrogenic Na pump in squid giant axons

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The sodium pump of animal cells is electrogenic[1,2], that is, it normally exports more sodium ions than it imports potassium ions[3]. In the squid giant axon, the resulting net outward electric current[4] has a density of a few μA cm^{-2}, and contributes 1–2 mV to the resting membrane potential[5,6]. The pump is driven by the free energy of hydrolysis of ATP, and in some instances[7–10] it has been possible to run the pump backwards and synthesize ATP by lowering the [ATP]/[ADP]·[P_i] ratio and steepening the transmembrane Na^+ and K^+ gradients. Here we have examined the question of whether a backward-running sodium pump conserves its $Na^+/K^+ > 1$ stoichiometry. We demonstrate reversal of the sodium pump of squid giant axon, and find that backward pumping indeed produces a net inward electric current. This current is voltage-sensitive. Our observations have mechanistic implications for models of the sodium pump.

It is generally accepted that the sodium pump normally operates with Na/K > 1 stoichiometry. What is less clear is whether this stoichiometry is fixed or variable and dependent on conditions such as steepness of the ion gradients, membrane potential and available free energy of ATP hydrolysis[2]. One extreme situation is a reversal of the pump's operation, resulting in synthesis of ATP. If the pump's normal stoichiometry (say, $3Na^+/2K^+$) were conserved, one should find an inward pump current. We have tested this hypothesis by forcing the sodium pump of squid giant axon to run backward, and simultaneously

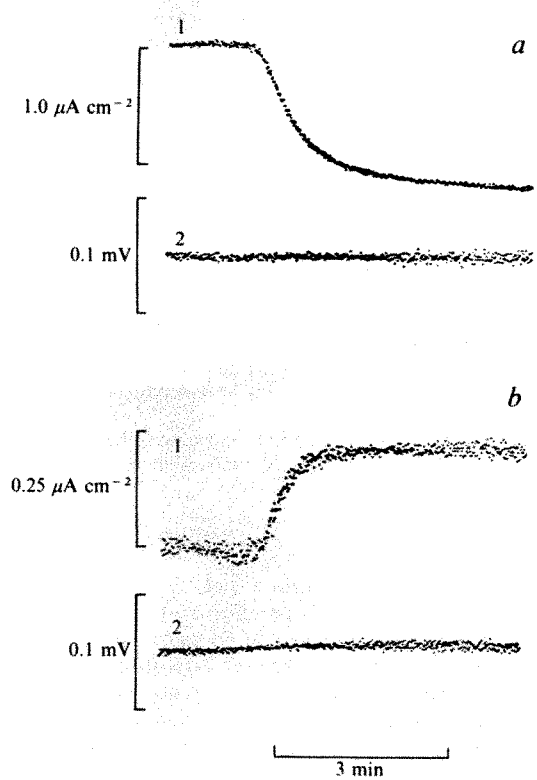


Fig. 1 Electrogenic pump measurements in squid giant axons. *a*, Axon bathed in artificial seawater containing (in mM) 425 NaCl, 10 KCl, 10 $CaCl_2$, 25 $MgCl_2$, 25 $MgSO_4$, 5 Tris-HEPES *p*H ~7.7 and 2×10^{-7} M tetrodotoxin, and internally dialysed with a solution containing (in mM) 50 NaCl, 200 K-glutamate, 100 K-HEPES *p*H 7.4, 250 glycine, 10 $MgCl_2$, 1 EGTA, 5 ATP, 3 phosphoarginine (PA) and 3 phosphoenolpyruvate (PEP), the latter four as potassium salts. 1, Holding current. About 1.3 min into the record, 10^{-5} M H_2DTG was added to the seawater. The resulting change in holding current density was -1.1 μA cm^{-2}. 2, Membrane potential. Holding potential varied less than 20 μV during the response. Temperature 17 °C. *b*, Axon bathed in K^+-free artificial seawater (NaCl replaced KCl). Internal fluid was Na^+-free (equimolar replacement with *N*-methyl-D-glucamine) and contained no ATP, PA or PEP, but 5 mM each of ADP and P_i, as well as 1 mM diadenosine pentaphosphate (an inhibitor of adenylate kinase) and 50 μM atractyloside (an inhibitor of mitochondrial ATP/ADP transfer). 1, Holding current; 2, membrane potential. The holding current change on addition of 10^{-5} M H_2DTG to the seawater was 0.23 μA cm^{-2}. The more rapid action of H_2DTG (compared with the forward case) can be explained by the absence of potassium from the seawater. Temperature 17 °C.

Methods: The axons were mounted in a Lucite chamber 9 × 3 × 3 mm, one side of which was a blackened platinum plate; the chamber was sealed at both ends with petroleum jelly. Artificial seawater superfused the axon at about 1 ml min^{-1}. A cellulose acetate capillary (diameter 140 μm; porous in its middle) was threaded through the axon, and a conventional 'piggy-back' voltage-clamp electrode assembly, made of a 100–120 μm, 0.5 M KCl-filled glass microelectrode with a 75 μm blackened platinum wire glued to it, was placed alongside the capillary and centred in the chamber. The current-passing platinum wire protruded 1–2 mm beyond the end of the chamber. The flow rate of the internal dialysis fluid was ~1 μl min^{-1}. A glass capillary electrode, similar to the internal one, was dipped into the chamber. Both electrodes were connected, via matched calomel electrodes, to a 'slow' (time constant 0.1 s) and very stable voltage-clamp device. When a steady perfusion regime had been achieved, the axon was voltage-clamped, usually at −60 mV or at its resting potential. Both holding potential and clamp current were digitized, collected and stored by a microcomputer. Ten data points were sampled and averaged every 825 ms for each point displayed in the figure. Outward holding current is displayed upward. To measure the magnitude of the change in holding current, straight lines were fitted by least-squares analysis to the linear portions of the record before and after the transient, and the vertical distance between the two lines was evaluated at the midpoint of the transient.

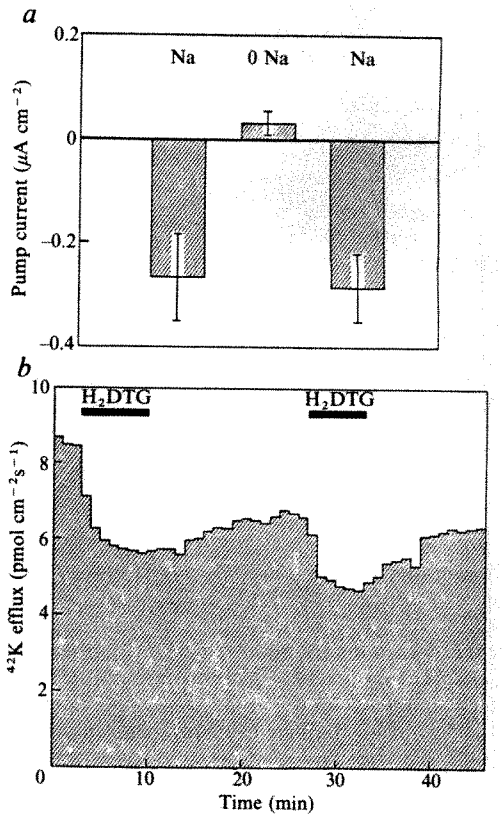


Fig. 2 Electrogenic current and potassium efflux during reverse pumping. *a*, Average (±s.e.m.) pump current in the inward direction (combined results of six tests on five axons) before (-0.27 ± 0.08), during ($+0.03 \pm 0.02$) and after (-0.29 ± 0.06 μA cm^{-2}) removal of sodium from the external seawater (replacement, *N*-methyl-D-glucamine). Bars show s.e.m. Sodium-free internal fluid throughout, as in Fig. 1*b*. Temperature 17 °C. *b*, Inhibition of ^{42}K efflux by H_2DTG. The effect of adding 10^{-5} M H_2DTG on ^{42}K efflux is shown for an axon held at −60 mV. Solutions as in Fig. 1*b*. The time during which H_2DTG was present in the external solution is indicated by the horizontal bar. A second application and washout of H_2DTG is also shown. The magnitude of the first response was 3.4 pmol cm^{-2} s^{-1}, and of the second, 2.0 pmol cm^{-2} s^{-1}. Temperature, 17 °C.

measuring cardiotonic steroid-sensitive membrane current under voltage-clamp control.

Giant axons from the hindmost stellar nerve of the squid, *Loligo pealei*, were cleaned, mounted in a horizontal chamber and superfused with artificial seawater at either 17 or 24 °C. The axons' intracellular composition was controlled by internal dialysis using a porous capillary[11]. Membrane potential was controlled by a slow and very stable voltage-clamp apparatus (R.F.R. *et al.*, in preparation). The principle of the pump current measurement[12] is as follows: sudden exposure of the voltage-clamped cell to a cardiotonic steroid (ouabain or the more reversible[4] dihydrodigitoxigenin, H_2DTG) will cause a change in clamp holding current exactly equivalent to the current generated previously by the sodium pump, provided that the steroid does not alter the passive membrane conductance. This requirement has been tested and is met (P. De W. *et al.*, in preparation).

Figure 1*a* shows a representative forward pump-clamp experiment, performed on an axon superfused with 425 mM Na^+, 10 mM K^+ seawater and internally dialysed with a solution containing 50 mM Na^+, 290 mM K^+ and 5 mM ATP. Addition of 10^{-5} M H_2DTG to the seawater produced an inward change in holding current (1.1 μA cm^{-2}). (For comparison, typical peak sodium channel membrane current densities in squid axon are ~2–5 mA cm^{-2}, and peak gating current densities, ~30 μA cm^{-2}.) The axon in Fig. 1*b*, on the other hand, was bathed in K^+-free seawater and internally dialysed with Na^+-

Table 1 Cardiotonic steroid-sensitive currents and fluxes in squid axons measured in conditions designed to produce reverse sodium pumping

Measurement	Mean ± s.e.m.	Membrane potential (mV)	No. of expts	No. of axons	Remarks*
Current ($\mu A\ cm^{-2}$)	-0.332 ± 0.037	-65.8 ± 1.7	19	9	17 °C; 1982
	-0.145 ± 0.014	-58.2 ± 1.5	32	12	17 °C; 1983
	-0.316 ± 0.058	-53.1 ± 1.5	11	4	24 °C; 1983
Sodium influx (pmol $cm^{-2}\ s^{-1}$)	6.38 ± 0.70	-55.5 ± 9.5	2	2	17 °C; 1982/83
Potassium efflux (pmol $cm^{-2}\ s^{-1}$)	2.50 ± 0.25	-57.4 ± 1.2	12	6	17 °C; 1983

All current measurements were carried out as described in Fig. 1 legend. The seawater was K^+-free as described for Fig. 1*b*; the cardiotonic steroid was either 10^{-5} M H_2DTG (reversible) or 10^{-5} M ouabain (irreversible). The internal dialysis fluid composition was as in Fig. 1*b*, except that [ADP] and [P_i] varied between 1 and 5 mM, with no obvious repercussions, and diadenosine pentaphosphate levels varied between 0.25 and 1 mM. Flux measurements were made in identical conditions except that either the seawater contained ^{22}Na or the internal dialysis fluid contained ^{42}K. Corresponding dialysis fluid or superfusate samples were collected and counted. Most of the ^{42}K experiments were conducted in the presence of 1 mM 3,4-diaminopyridine, which blocks potassium channels[19].

* For unknown reasons, perhaps related to ocean temperatures or other environmental factors, the 1982 animals appeared to have higher reverse sodium pump activity than the 1983 specimens. The forward pumping rate was also higher in the 1982 animals. A comparable year-to-year variation has previously been seen in the case of Mg^{2+} extrusion by these cells[20].

free fluid containing 5 mM ADP, 5 mM P_i and no ATP (ATP formation was blocked by diadenosine pentaphosphate and atractyloside). Here H_2DTG caused an outward change in holding current (0.23 $\mu A\ cm^{-2}$), signifying the disappearance of an inward pump current. Table 1, summarizing 60 reverse pump current measurements obtained on 30 axons, suggests a considerable temperature coefficient for reverse pumping ($Q_{10} \approx 3$). The presumption that Fig. 1*b* truly represents inhibition of a reverse electrogenic sodium pump (and not of, say, a forward pump with $K^+ > Na^+$ stoichiometry) was corroborated in three ways. First, a ouabain-sensitive sodium influx was seen in axons dialysed with Na^+-free fluid containing ADP and P_i (Table 1). Pump-mediated Na^+ influx into high-ADP cells is well known, but only as part of electroneutral $Na^+:Na^+$ exchange[4,12,13], which obviously requires intracellular Na^+. Second, the putative inward pump current is abolished by removal of Na^+ from the seawater (Fig. 2*a*). Third, we measured a cardiotonic steroid-sensitive ^{42}K efflux (Table 1, Fig. 2*b*) into K^+-free seawater, a condition which precludes $K^+:K^+$ exchange, a known mode of operation of the sodium pump[14,15]. (In unclamped axons engaged in forward pumping, ouabain causes ^{42}K efflux to increase through activation of K^+ channels following the depolarization which results from inhibition of the electrogenic sodium pump[6]). Simultaneous ^{42}K efflux and inward pump current measurements were made in 11 instances on 7 axons. The average (K^+ flux)/(net current) ratio was 2.7 ± 0.5, or one elementary charge carried in for every 2–3 K^+ ions pumped out. This suggests a Na^+/K^+ stoichiometry of 3/2 or 4/3.

A remarkable feature emerges when reverse electrogenic pump current is plotted against membrane potential (Fig. 3). In five cases from four axons, pump current measurements at a given potential were combined with measurements at another potential, and without exception, revealed a strong voltage dependence. Interestingly, the voltage dependence represents a negative pump conductance: contrary to simple thermodynamic expectation, the inward current decreases as the cell is made more negative. This may mean either that the pump operates in series with a voltage-sensitive gate[16] or that a rate-limiting kinetic step in the pump cycle is inhibited by negative membrane potentials[17,18].

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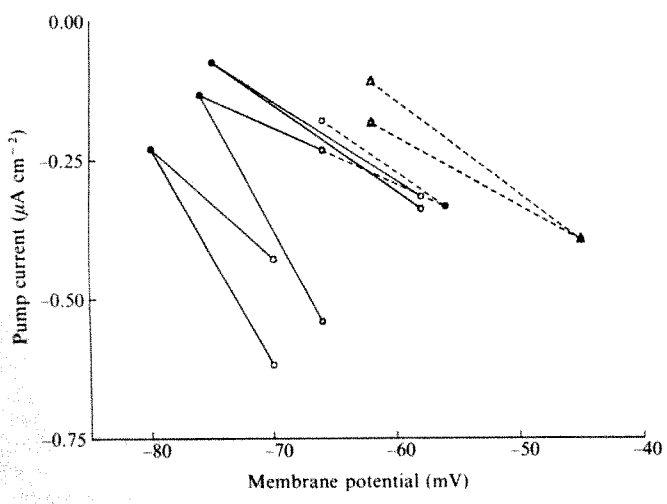


Fig. 3 Relationship between reverse pump current and membrane potential. Measurements were made as described in Fig. 1 legend. All axons were exposed to K-free seawater and perfused as described for Fig. 1*b*. Circles, 1982 data; triangles, 1983 data. The solid and dashed lines connect sets of bracketing (open symbols) and bracketed (filled symbols) measurements. Solid lines, bracketed test at hyperpolarized potential; dashed lines, bracketed test at depolarized potential. At each control voltage 10^{-5} M H_2DTG was applied, then washed out; the holding potential was changed to the test voltage during the recovery, and the axon re-exposed to H_2DTG at the test voltage; finally, during recovery from the second H_2DTG application the holding potential was changed back to the original control voltage, and the axon was exposed to H_2DTG for the third time. Temperature, 17 °C.

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Mechanism of ion permeation through calcium channels

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Calcium channels carry out vital functions in a wide variety of excitable cells[1-4] but they also face special challenges. In the medium outside the channel, Ca^{2+} ions are vastly outnumbered by other ions. Thus, the calcium channel must be extremely selective if it is to allow Ca^{2+} influx rather than a general cation influx. In fact, calcium channels show a much greater selectivity for Ca^{2+} than sodium channels do for Na^+ (refs 5–9) despite the high flux that open Ca channels can support[3,4]. Relatively little is known about the mechanism of ion permeation through Ca channels. Earlier models assumed ion independence[5] or single-ion occupancy[10–16]. Here we present evidence for a novel hypothesis of ion movement through Ca channels, based on measurements of Ca channel activity at the level of single cells or single channels. Our results indicate that under physiological conditions, the channel is occupied almost continually by one or more Ca^{2+} ions which, by electrostatic repulsion, guard the channel against permeation by other ions. On the other hand, repulsion between Ca^{2+} ions allows high throughput rates and tends to prevent saturation with calcium.

Single ventricular cells were obtained from adult guinea pigs by enzymatic dissociation[17] and a suction pipette was used for voltage clamp and internal dialysis[18]. To minimize current flow through channels other than the Ca channel, internal and external solutions were kept Na^+- and K^+-free[6].

Figure 1*a* shows how the Ca current (I_{Ca}) varies with the bathing calcium concentration ($[Ca]_0$). The peak magnitude of I_{Ca} increases almost proportionally with $[Ca]_0$ over the range 1–10 mM, with increasing saturation as $[Ca]_0$ approaches 30 mM. This type of $[Ca]_0$ dependence is similar to that found for Ca channels in cardiac preparations and other excitable cells[1,2,11–16]. The saturation of I_{Ca} has been attributed to a single divalent cation-binding site within the Ca channel with a dissociation constant for Ca^{2+} (K_{mCa}) of ~10 mM (refs 11–16).

The interaction between Ca^{2+} and the channel can also be gauged by using Ca^{2+} to block the movement of other ions through the Ca channel. As Fig. 1*b* illustrates, addition of 0.6 mM Ca_0 to the bathing medium reduced the inward current in the presence of 10 mM Ba to less than half; in four cells the average decrease of current was to 41 ± 4% of that in the absence of Ca. The Ca-sensitivity of Ba currents is similar to the Ca-sensitivity of Sr action potentials in cardiac Purkinje fibres[10].

Figure 1*c* shows that Ca channel current carried by Na^+ (150 mM) is even more sensitive to $[Ca]_0$. Addition of only 1.3 μM Ca to the bathing medium lowered the Na influx through the channel to 33 ± 4% in three cells. This result is consistent with previous work in heart[19], snail neurones[14], skeletal muscle[20] and other excitable cells[13].

The very different estimates of K_{mCa} in Fig. 1*a–c* are inconsistent with competition for a single binding site. The discrepancy between various estimates of K_{mCa} would be even greater if allowance were made for changes in surface potential and binding of other ions. The estimate of K_{mCa} from Fig. 1*a* would be revised upward if allowance were made for effects of surface potential on the degree of Ca channel opening. On the other hand, estimates of K_{mCa} from Fig. 1*b* and *c* would have to be revised downward if binding of Ba or Na were taken into account.

The extreme $[Ca]_0$-sensitivity of Na movements through the Ca channels has been attributed to a high-affinity, regulatory Ca-binding site outside the channel, distinct from a relatively low-affinity Ca-binding site that lies within the channel and controls ion permeation[16]. Figure 2 shows another test of the one-site model which leads us to a different interpretation of

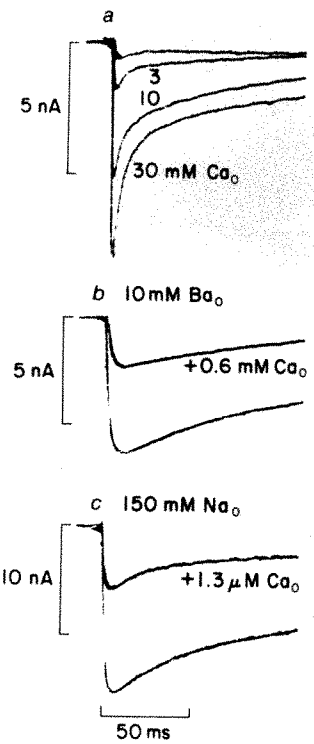


Fig. 1 Comparison of the ability of extracellular Ca to act as a charge carrier through the Ca channel (*a*) with its ability to block current through the channel carried by other ions (*b*, *c*). All records show net ionic currents after subtraction of linear leak and capacity currents determined from hyperpolarizing pulses. *a*, Currents recorded in consecutive runs with 1, 3, 10 and 30 mM $[Ca]_0$. Depolarizing pulses to +20 mV from a holding potential of −40 mV. External and internal solutions were Na^+- and K^+-free. Cell 165C. The internal solution contained (in mM): 75.5 Cs-aspartate, 75.5 Cs-phosphate, 10 Cs-EGTA, 6 HEPES (*p*H 7.4). The external solution contained (in mM): 85 Cs-aspartate, 146 sucrose, 2.8 $MgCl_2$, 5 glucose, 5 HEPES (*p*H 7.4) and $CaCl_2$ or $BaCl_2$ as noted in the figures. Series resistance compensation was used to offset the ohmic drop resulting from current flow through the tip of the suction pipette. Current signals were recorded and analysed on a PDP 11-03 laboratory computer. All experiments were done at room temperature (21 °C). *b*, Block of Ba current through Ca channels by addition of external Ca. Records shown were obtained with 10 mM $[Ba]_0$ in the absence and presence of 0.6 mM $[Ca]_0$. Voltage clamp pulse from −40 mV to +20 mV. Cell 180N. *c*, Block of Na current through Ca channels by addition of external Ca. The control record was obtained with the standard internal solution (see text) and the following external solution: 150 mM NaCl; 2 mM EGTA, 12.5 μM tetrodotoxin (TTX), 10 mM HEPES (*p*H 7.6). $[Ca]_0$ was then increased to 1.3 μM by changing to the following solution: 150 mM NaCl, 2 mM total HEDTA, 1 mM total Ca, 12.5 μM TTX, 10 mM HEPES (*p*H 7.6, apparent stability constant for CaHEDTA taken as $7.6 \times 10^5\ M^{-1}$). Pulse from −60 mV to +10 mV. Cell 207H.

the various effects of Ca^{2+}: *a* shows the behaviour expected for a simple one-site channel. In mixtures of Ca and Ba in which $[Ca]_0 + [Ba]_0$ is held constant at 10 mM, the total current can never be smaller than that in either 10 mM $[Ca]_0$ or $[Ba]_0$ alone; the total current must be a monotonic function of the mole fraction. This prediction was tested experimentally with recordings of whole-cell current in guinea pig ventricular myocytes (Fig. 2*b*). In contrast to the properties expected for a simple one-site channel, the measured current is not a monotonic function of the mole fraction, $[Ba]_0/([Ba]_0 + [Ca]_0)$, but goes through a clear minimum as 70% of the external Ca is replaced by Ba. Similar behaviour of currents in mixtures of permeant ions has been reported previously for gramicidin channels[21,22], K channels[23] and Cl channels[24,25]. Such 'anomalous mole fraction effects'[26] have been accounted for by models which include two (or more) ion-binding sites within the channel and repulsive

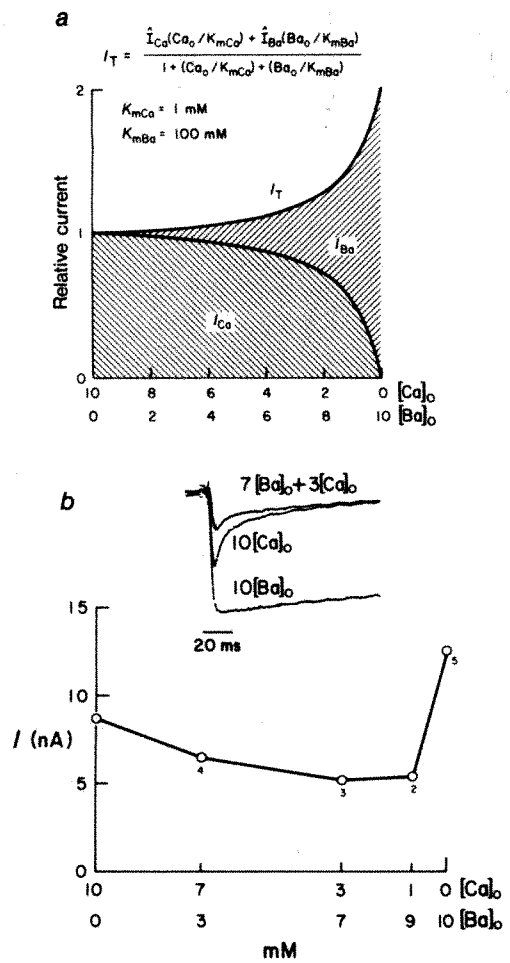


Fig. 2 The mole-fraction dependence of Ca channel current predicted for a channel with one ion-binding site (*a*) compared with that found experimentally (*b*). *a*, Computed values of total current (I_T) and its components I_{Ca} and I_{Ba}, calculated for a one-site channel using the equation shown. The dissociation constants of the site for Ca and Ba (K_{mCa} and K_{mBa} respectively) were chosen as shown in the inset. The maximum Ba current ($\hat{I}_{Ba}$) was made twice as large as the maximum Ca current ($\hat{I}_{Ca}$) because Ba currents are larger than Ca currents in these cells. No matter what values are chosen for K_{mCa}, K_{mBa}, $\hat{I}_{Ca}$ and $\hat{I}_{Ba}$, the one-site model always predicts that I_T is a monotonic function of the mole fraction. *b*, Peak amplitude of leak-subtracted inward current plotted against mole fraction. Voltage clamp pulse from −40 mV to +10 mV. To ensure that the anomalous mole-fraction dependence was not the result of rundown, the solutions were applied in the order indicated by the numbers next to each data point. Individual current records are shown in the inset. Cell 166A.

interactions between ions in the doubly occupied state[27,28].

The main uncertainty concerning the results in Fig. 2 is that changes in the peak current may not reflect changes in the current through open channels (*i*) but rather changes in the probability of channels being open (*p*). To measure single-channel current, we performed ensemble fluctuation analysis[29] on whole-cell recordings[30] from single frog ventricular cells[31]. Figure 3 shows a determination of *i* during the inactivation phase of Ca channel current. Estimates of *i*, the number of functional channels (N_F), and the peak probability of channel opening (p_{max}) are in reasonably good agreement with results of ensemble fluctuation analysis during the rising phase of Ca channel current[31]. As Fig. 3*c* shows, the main point here is that with $[Ca]_0 = 3$ mM and $[Ba]_0 = 7$ mM, *i* is significantly smaller than with $[Ca]_0 = 10$ mM or $[Ba]_0 = 10$ mM. Similar results were obtained in rat ventricular cells at a test potential of +20 mV (B. P. Bean and M. C. Nowycky, unpublished).

To explore the implications of the anomalous mole-fraction effect, we modelled the Ca channel as a single-file pore with

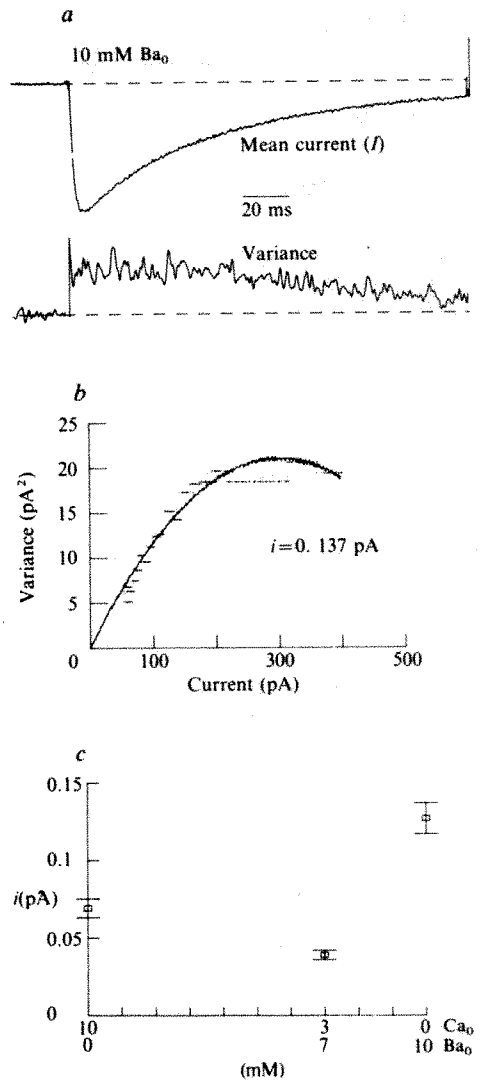


Fig. 3 The anomalous mole-fraction effect is a property of the open Ca channel. Single-channel currents were estimated by non-stationary fluctuation analysis[24] as illustrated in *a* and *b*. Whole-cell currents were recorded from single frog ventricular cells using the patch-clamp technique[25]. Fifty consecutive current records obtained with voltage steps from −60 mV to +10 mV were lined up in time and the mean and variance were calculated for each sampling point. The time course of the mean current (after subtraction of linear leak and capacity currents) and the variance are shown in *a*. The variance associated with the holding current was taken as the baseline (dashed line). *b*, Plot of the variance associated with the falling phase of the current as a function of the mean current. The data are fitted to the equation: variance = $iI - I^2/N_F$, where $I = N_F iP$. These equations describe the behaviour of N_F independent channels with a single-channel current *i* and a probability *P* of being open[29]. For the example shown, the best-fit parameters were $i = 0.137$ pA, $N_F = 4{,}340$ channels and $P_{max} = 0.66$. The external solution contained (in mM:): $BaCl_2$ 10, tetraethylammonium-Cl 135, $MgCl_2$ 1, HEPES 10 (*p*H 7.6). The patch pipette contained (in mM): CsCl 120, $MgCl_2$ 5, EGTA 10. Cell P11B. *c*, Mean (±s.e.m.) single-channel currents at a test potential of +10 mV determined by the method described above in 10 mM $[Ca]_0$ ($n = 7$ cells), 7 mM $[Ba]_0$ + 3 mM $[Ca]_0$ ($n = 6$) and 10 mM $[Ba]_0$ ($n = 7$).

two ion-binding sites (energy wells) and three energy barriers (for example, see refs 27, 28, 32). Figure 4*a* shows the various states of ion occupancy for the case of two permeant cations (for example, Ca and Ba). Rates of transition between connected states were calculated from Eyring rate theory, with energy profiles for Ca and Ba like those shown in Fig. 4*b*. For simplicity, the ion-binding sites (energy wells) were assumed to be equally spaced within the membrane; symmetry of energy profiles was

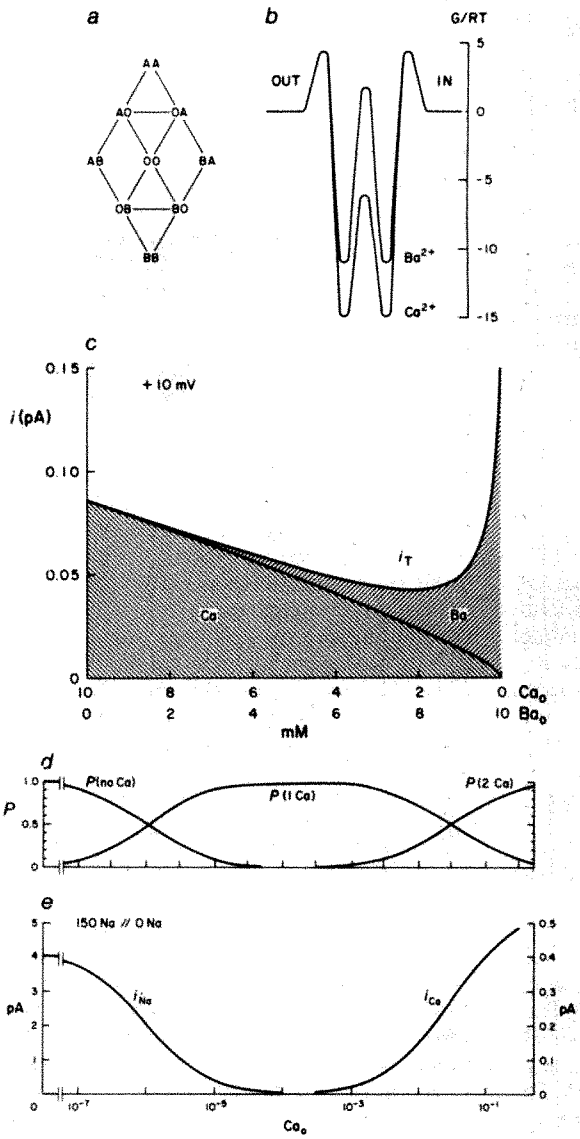


Fig. 4 Two-binding site model for the calcium channel. *a*, State diagram for the case of two ions. A and B, present on both sides of the membrane. An empty site is denoted by O. As the diagram indicates, single-filing is assumed. *b*, Free energy profiles for a single ion (Ca^{2+} or Ba^{2+}) in the absence of both an applied electric field and other ions within the channel. To reduce the number of adjustable parameters, barriers and wells are assumed to be spaced equally, 1/6th of the membrane thickness apart; chemical energy profiles are assumed to be symmetrical with respect to the centre of the membrane. As in earlier treatments of this type (see ref. 28 for earlier references), rates of transition were calculated by Eyring rate theory expressions of the form rate $= Q \exp(-\Delta G)$ (for entry rates this expression is multiplied by the ion activity in the medium from which the ion enters the channel). By convention, $Q = (kT\,h^{-1})$ as in treatments of ion permeation through Na and K channels[28,32,34]. The free energy change in surmounting a barrier ΔG is taken as the sum of three terms: a purely chemical ΔG^0 (as indicated in *b*), an electrical term $\pm(z_i V/6)$, and (when the far site is occupied) a repulsion term, $\pm z_i z_j \ln(F_{mm})$, where $F_{mm} = 3$ (z = valence and V = membrane potential). The choice of signs was determined by the direction of the transition relative to the applied electric field and to the position of the repelling ion. *c*, Calculated anomalous mole-fraction effect with $[Cs]_0 = 85$ mM and $[Cs]_i = 171$ mM (as in Fig. 2*b*), $[Ba]_i = 0$ and $[Ca]_i = 0$ at +10 mV. (Calculations with a computer model based on a program kindly provided by Dr T. Begenisich.) Activity coefficients for monovalents (divalents): 0.764 (0.341) outside and 0.725 (0.276) inside. Chemical component of energy profile for Cs^+ (in units of RT, where R is the gas constant and T is the absolute temperature): wells, −6, −6; barriers from outside to inside, 7.8, 10.3, 12.8. This profile also fits measured Ca channel reversal potentials with $[Ba]_0 = 10$ mM or $[Ca]_0 = 10$ mM and internal Cs[9,35]. Ca and Ba components of net current are shown; as Cs flux is less than 1.5% of net current magnitude, it is not shown. *d*, Occupancy of the channel by Ca as a function of $[Ca]_0$. Probabilities of the channel containing 0,1 or 2 Ca ions are shown at 0 mV in the presence of 150 mM external Na and no permeant internal ions. The chemical component of the energy profile for Na^+ (in units of RT: wells 1,1; barriers 6,8,10 from outside to inside) also fits measured reversal potentials of $[Na]_0$ versus $[Cs]_i$ and $[Ba]_0$ versus $[Na]_i$ (unpublished results). *e*, Elementary inward currents computed for the conditions assumed in *d*. At $[Ca]_0 < 10^{-4}$ M, inward current is carried by Na (i_{Na}); Ca only carries significant current at $[Ca]_0 > 10^{-4}$ M (i_{Ca}).

assumed in the absence of compelling reasons to the contrary. Ion–ion interactions were incorporated by a repulsion factor F which has the effect of making entry rates into a site F-fold slower and exit rates F-fold faster if the other site is occupied by another cation[28]. For monovalent–monovalent (mm) interactions, F_{mm} was set at 3 (well within the range expected for reasonable assumptions about the dielectric environment surrounding an aqueous pore[33]). For monovalent–divalent and divalent–divalent (dd) interactions, F was given by Coulomb's law without additional adjustable parameters: thus $F_{dd} = (F_{mm})^{2\cdot2} = 3^4 = 81$. Once specific values for the barrier heights and well depths are assigned to each ion, the model allows the computation of ion fluxes and ion occupancies in the presence of three permeant ions (for example, Ca, Ba and Cs).

A central feature of the model is that both binding sites have a high affinity for Ca ions. The postulated well depth for Ca of −15RT corresponds to an apparent dissociation constant of $\exp(-15) = 3 \times 10^{-7}$ M, three or four orders of magnitude lower than earlier estimates[10–16]. With the barrier heights and well depths shown in Fig. 4*b*, the model can account for the present experimental results: (1) calculated unitary currents exhibit an anomalous mole-fraction dependence (Fig. 4*c*); (2) Na movements through the channel are blocked by micromolar levels of Ca (Fig. 4*e*); while (3) Ca current increases as $[Ca]_0$ is elevated, with little saturation until $[Ca]_0$ reaches several millimolar (Fig. 4*e*). According to the model, these observations may be interpreted as follows.

The anomalous mole-fraction effect arises because the channel binds Ca^{2+} more strongly than Ba^{2+} and because Ca^{2+} and Ba^{2+} ions repel each other within the channel (see refs 27, 28). When the inner site is occupied by a Ca ion, a Ba ion entering across the outer barrier is very quickly repelled back out of its shallower well. Thus, equimolar replacement of external Ca by external Ba makes the current smaller at first because Ba^{2+} remains relatively impermeable until $[Ca]_0$ is low.

As Ca^{2+} binds so strongly, channel occupancy by a single Ca^{2+} is enough to block Na flux through the channel, and this occurs at micromolar levels of $[Ca]_0$. On the other hand, Ca flux through the Ca channel increases hand-in-hand with the probability of two Ca^{2+} ions in the channel (Fig. 4*d*, *e*). Because of mutual repulsion, this probability only becomes significant at millimolar $[Ca]_0$. Once double occupancy has occurred, repulsion promotes current flow. The presence of a Ca^{2+} at the outer site repels a Ca^{2+} at the inner site, increasing its chances of leaving that site and entering the cell. In this way, channel occupancy by Ca^{2+} tends to perpetuate itself, and Ca^{2+} strongly reinforces its own permeability. Only a second Ca^{2+} stays long enough to have a significant chance of displacing a predecessor Ca^{2+}. Like Ba^{2+}, Na^+ and other ions which bind less strongly than Ca^{2+} are quickly repelled out of the channel by Ca^{2+}.

This explanation helps resolve the controversy about the ionic basis of the 'slow inward current' (I_{si}) in cardiac muscle. On one hand, I_{si} has been called I_{Ca^{2+},Na^+} because Na^+ ions can support Mn^{2+}- and verapamil-sensitive action potentials or inward currents in the absence of external Ca[19]. On the other hand, in the presence of external Ca, prolonged removal of external Na reduces I_{si} very little[7,8]. Our results suggest that there is no contradiction between the channel's ability to support a large Na influx in the absence of Ca and its ability to reject Na in the presence of Ca. The high transport number for Ca in

physiological conditions justifies the simple label 'Ca channel' in the same way that the Na selectivity of tetrodotoxin (TTX)-sensitive channels justifies the name 'Na channel'.

This report is the first to argue that Ca channels are multi-ion, single-file pores, rather than one-ion pores that function by simple mutual exclusion. Our model is very different from the two-site model recently proposed by Kostyuk *et al.*[16]—they assumed that the pore can only contain one ion at a time and concluded that the channel selects for Ca over Ba at its outer binding site with $K_{mCa} = 10$ mM and $K_{mBa} = 100$ mM. To explain Ca^{2+} block of Na^+ influx, they had to postulate an additional external regulatory site with high affinity for Ca which controls the structure of the pore. Their model cannot account for the anomalous mole-fraction effect and it predicts that block of Ba currents by Ca^{2+} would require a $[Ca]_0$ at least 10 times higher than that described here.

As multi-ion pores, Ca channels resemble several other channels that are selective for Na, K or Cl; however, the proposed selectivity mechanism differs from previous descriptions of other biological channels. Unlike Na channels[32,34], Ca channels seem to bind the preferred ion more strongly than other physiological ions (see also refs 11, 16). Ca is selected as much by deep-energy wells as by a relatively low-energy barrier ('selectivity filter'). The nature of the binding site is of obvious importance. It is interesting to compare the Ca channel with Ca binding proteins such as troponin and calmodulin, or small organic Ca buffers such as EGTA. Like the proposed Ca binding sites in our model channel, the Ca-specific coordination sites on these molecules bind Ca^{2+} with high affinity (effective $K_d < 1$ μM), in preference to other cations (for example, Ba^{2+}, Sr^{2+}, Mg^{2+}, Na^+). Although detailed differences are to be expected, it seems possible that Ca channels and intracellular Ca receptors may share similar mechanisms of Ca selectivity.

After submission of this manuscript and publication of preliminary communications of this work[35,36], we received a manuscript with a similar theoretical treatment of Ca channels in skeletal muscle by W. Almers and E. W. McCleskey[37].

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Complete tyrosine-*O*-sulphation of gastrin in neonatal rat pancreas

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Modification of tyrosine residues of proteins has attracted great interest. Although phosphorylation has received most attention[1–3], tyrosine-*O*-sulphation is also common[4] and has been closely investigated in the hormones gastrin and cholecystokinin (CCK). CCK regulates pancreatic enzyme secretion. It is always sulphated[5–9] and sulphation increases its pancreatic secretagogue activity 100-fold[10–13]. By contrast, only half of gastrin molecules are tyrosine-*O*-sulphated[5–9] and sulphation does not influence the ability of gastrin to regulate gastric acid secretion[6]. We now report that in neonatal rat pancreas, which is the major source of gastrin in the newborn rat[14], gastrin is completely sulphated. Moreover, the disappearance of gastrin from the pancreas during the first weeks after birth parallels the appearance of CCK in the intestine. Since sulphation is essential for pancreatic secretagogue activity of gastrin as well as of CCK[10–13], pancreatic gastrin in the neonate may be the equivalent of intestinal CCK in the adult.

The pancreas, antrum and small intestine of 0-, 4-, 7-, 14- and 21-day-old Wistar rats and of 17-day fetuses were dissected out and frozen immediately on dry ice. Tissues were extracted by boiling in water and the pellet was re-extracted in cold 0.5 M acetic acid. The concentrations of gastrin and CCK in the extracts were measured by sequence-specific radioimmunoassays (RIAs), including one which distinguishes between non-sulphated and sulphated peptides[9,15–17]. The extracts were further analysed by Sephadex G-50 superfine chromatography and ion-exchange HPLC (see Fig. 3 legend). Both gel and ion-exchange chromatography were monitored using gastrin RIAs, one (using antiserum no. 2604) measuring sulphated and non-sulphated gastrins[15] and another (using antiserum no. 2605) measuring only non-sulphated gastrins[9,16].

Figure 2 shows the fetal and postnatal changes in pancreatic gastrin and intestinal CCK concentrations. At birth, pancreatic gastrin concentration was high whereas gastrin and CCK concentrations in the small intestine were low, relative to adult concentrations. After the fourth postnatal day, pancreatic gastrin rapidly disappeared, concomitant with the appearance of CCK in the intestine.

Gel chromatography of the pancreatic extracts followed by RIA using the antiserum that binds both sulphated and non-sulphated gastrins revealed only one peak of immunoreactivity, in a position corresponding to 'little' gastrin (or gastrin-17), and which was not recognized by the antiserum that binds only non-sulphated gastrins. The pancreatic and antral little gastrin was further characterized by high-pressure ion-exchange chromatography of the gastrin-containing fractions from the gel chromatography. By contrast with antral gastrin which was partially sulphated, gastrin from the neonatal pancreas was completely sulphated. Gastrin immunoreactivity in the pancreas co-eluted with both sulphated antral gastrin immunoreactivity (Fig. 3*a*, *b*) and biosynthetically ^{35}S-sulphate-labelled gastrin (Fig. 3*a*, *c*). Moreover, pancreatic gastrin was desulphated by treatment with limpet arylsulphatase (Fig. 3*d*).

Antral little gastrin was resolved by ion-exchange chromatography into four peaks (Fig. 3*b*), probably representing sulphated and non-sulphated forms of gastrin-15 and -17, since rat gastrin-17 contains arginine (Fig. 1) one amino acid after the dibasic cleavage site of gastrin-17 (ref. 18). These identifications were confirmed by the observation that on ion-exchange chromatography of trypsin-treated antral gastrin, there was a single peak for each of sulphated and non-sulphated gastrin

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MURINE GASTRIN-17 pGLU-ARG-PRO-PRO-MET-GLU-GLU-GLU-GLU-GLU-ALA-[SO_3^-]TYR-GLY-TRP-MET-ASP-PHE·NH_2

PORCINE GASTRIN-17 pGLU-GLY-PRO-TRP-MET-GLU-GLU-GLU-GLU-GLU-ALA-[SO_3^-]TYR-GLY-TRP-MET-ASP-PHE·NH_2

PORCINE CCK-17 -ASP-PRO-SER-HIS-ARG-ILE-SER-ASP-ARG-ASP-[SO_3^-]TYR-MET-GLY-TRP-MET-ASP-PHE·NH_2

Fig. 1 The primary structure of heptadecapeptide gastrin from rat[18], pig[27] and of the C-terminal heptadecapeptide fragment of pig CCK[5]. The heptadecapeptide amide is the principal hormonal form of gastrin. The homologous C-terminal sequences containing the biologically active sites of both gastrin and CCK are underlined. The sulpho-tyrosine groups of the gastrins are given in brackets, since only half of the gastrin-17 molecules in rat and pig antral mucosa are sulphated, in contrast to the completely sulphated porcine CCK.

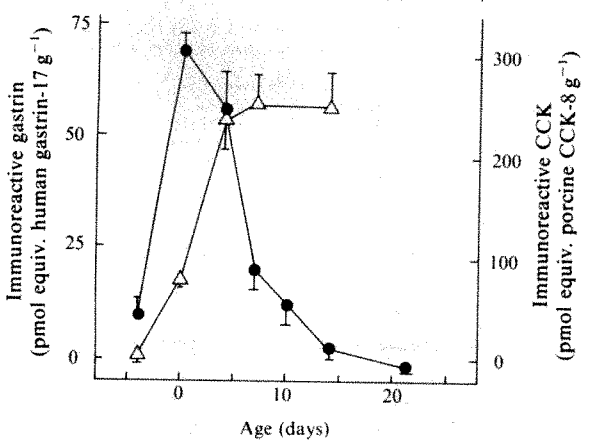


Fig. 2 The neonatal changes of gastrin in the pancreas (●) and CCK in the small intestine (△) of the rat. The number of rats for each point is 20 pooled in five groups of 4. Gastrin was measured in aqueous extracts with a specific RIA using a rabbit antiserum (2604; ref. 15), which detects sulphated and non-sulphated gastrins with equimolar potency, but CCK negligibly ($P < 0.0006$). CCK was measured in both aqueous and acetic acid extracts of intestine in a specific CCK RIA[17], using a rabbit antiserum (G-160; ref. 28) with which sulphated gastrins react at less than 0.005% and non-sulphated gastrins not at all.

(data not shown). Most of the pancreatic gastrin co-eluted with the trypsin-labile peak of little gastrin extracted from rat antrum and is thus presumably gastrin-17, although a small peak of sulphated gastrin-15 was also seen (Fig. 3*a*). The proximal small intestine contains moderate amounts of gastrin in the fetal, neonatal and adult rat (ref. 14 and this study). In both adult and neonate, however, this intestinal gastrin was similar to antral gastrin in being only partially sulphated.

Although the physiological significance of the transient expression of gastrin in the neonatal pancreas is not yet clear, the fact that it is completely tyrosine-*O*-sulphated suggests a CCK-like action on surrounding exocrine cells by a paracrine mode of release. Tyrosine-*O*-sulphation of CCK increases pancreatic acinar receptor binding and secretagogue potency by 100-fold[11,12]. Although sulphated gastrin is a weaker stimulus than sulphated CCK, this would be compensated for by the higher concentrations achieved through intrapancreatic release. Birth heralds a transition from placental to alimentary nutrition, so the dramatic changes in pancreatic gastrin and intestinal CCK concentrations in the neonate (Fig. 2) would seem to be related to the start of feeding. Pancreatic gastrin levels rise just prior to birth, before food is present in the intestinal lumen. After birth, CCK appears in the intestine and pancreatic gastrin rapidly disappears as intestinal stimuli become more important in controlling pancreatic function. Pancreatic gastrin may therefore be important in stimulating rapid pancreatic development at birth to ensure adequate digestion when feeding commences.

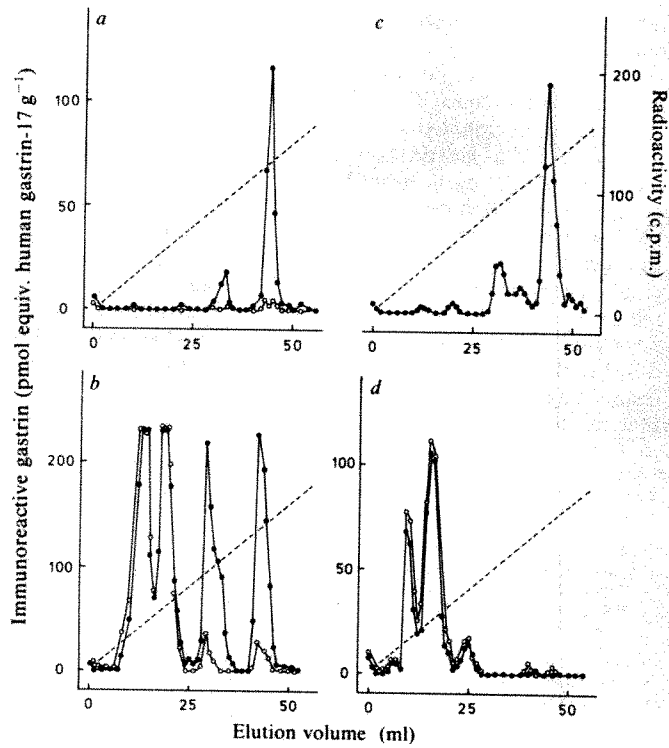


Fig. 3 *a*, Sulphation of gastrin in neonatal pancreas. Extracts were applied to a 5×50 mm Mono-Q HR5/5 anion-exchange column (Pharmacia) equilibrated with 20 mM sodium phosphate, *p*H 6.5. The column was washed with 10 ml 0.01% v/v acetic acid and eluted with a gradient from 0.01% to 12.5% acetic acid (----) over 50 min at room temperature with a flow of 1 ml min^{-1} (ref. 29). One-minute fractions were collected and assayed after removing the acetic acid by freeze-drying with two gastrin RIAs using antisera 2604 (●) and 2605 (○). *b*, Sulphation of gastrin in antral extracts fractionated on Mono-Q anion-exchange column as described above. Gastrin was measured by RIAs using antisera 2604 (●) and 2605 (○) as described above. *c*, ^{35}S activity (c.p.m.; ●) from slices of rat antrum incubated with ^{35}S-sulphate in sulphate-free Hank's media for 4 h. Antral extracts were then applied to a Seppak cartridge (Waters Associates), washed extensively, eluted with methanol, dried and applied to a Sephadex G50 column. The gastrin-17-like immunoreactivity was pooled and applied to a Mono-Q column and eluted as above and ^{35}S-sulphate radioactivity counted by liquid scintillation. Incorporation of ^{35}S-sulphate activity was directly confirmed by two-dimensional thin-layer electrophoresis of alkaline hydrolysates of the peptide[4]. All the ^{35}S-sulphate radioactivity co-migrated with tyrosine-*O*-sulphate standard. *d*, Pancreatic gastrin purified by gel chromatography was treated with limpet arylsulphatase (Sigma S8629). Dried peptide was dissolved in 800 μl of 0.2 M sodium acetate, *p*H 5, and 250 μg arylsulphatase, dissolved in 100 μl 0.2% sodium chloride, were added and the mixture incubated at 37 °C for 2 h. The incubation mixture was diluted with water and then applied to a Mono-Q anion-exchange column, eluted as above and gastrin measured by RIA using antisera 2604 (●) and 2605 (○).

In addition to stimulating pancreatic growth and secretion, gastrointestinal and pancreatic peptides also stimulate enzyme synthesis. Although this action is best understood for insulin, which selectively stimulates amylase gene transcription[19], CCK-like peptides may also stimulate trypsin and chymotrypsin synthesis[20]. These actions may be especially important in the neonate as just before parturition pancreatic enzyme concentrations rise markedly[21]. Pancreatic insulin[21] and gastrin concentrations (this study) also show parallel rises just before parturition, suggesting that these hormones induce enzyme synthesis in the surrounding exocrine cells. Whether the actual synthesis of pancreatic gastrin including the tyrosine-*O*-sulphation, is controlled by mechanisms inherent in the gastrin-producing cell

DNA or by signals from the environment, remains to be determined.

The complete sulphation of gastrin is not restricted to the neonatal rat pancreas. The feline pancreas contains gastrin that is completely sulphated and, although the normal human pancreas is devoid of gastrin, the small intestine of the human fetus contains 30-fold more gastrin than CCK and all the gastrin molecules are sulphated. After birth, the concentration of intestinal gastrin declines and gastrin assumes the adult partly-sulphated pattern (B.N.A., in preparation). Some human pancreatic gastrinomas contain only sulphated gastrin[22]. In view of the localization of essentially all malignant gastrinomas to the pancreas[23] and the homology between sulphated gastrin and the transforming middle-T antigen of polyoma virus[24], the present results may also be of relevance to oncogenesis. Note, however, that the homology is less pronounced in the sequence corresponding to the active site of gastrin, the C-terminal tetrapeptide amide.

It has previously been suggested that important changes can occur in peptide processing during neonatal development[25,26]. Our results support the contention that an organ at different stages of development can be regulated by different peptides through modulation of a post-translational modification critical for receptor binding.

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Expression and amplification of the N-*myc* gene in primary retinoblastoma

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Retinoblastoma, the most common intraocular tumour of childhood, probably arises from embryonal cells and occurs in hereditary and non-hereditary forms[1]. Recent evidence suggests that this retinoblastoma (*Rb*) susceptibility gene located at chromosome 13q14 is actually recessive[2–4]. Knudson has proposed that the tumour is caused by two mutational events[5]. This idea was extended by Comings[6], who suggested that dominantly inherited tumours may result from loss or inactivation of both alleles of regulatory or suppressor genes that, when active, prevent the expression of a structural transforming gene(s) (possibly an oncogene) normally active only during embryogenesis. Despite circumstantial evidence[3,4,7,8] for this hypothesis, no activated oncogene[9–14] has been identified. We now report that (1) the N-*myc* gene is amplified 10–200-fold in two primary retinoblastomas and a retinoblastoma cell line Y79 and (2) expression of N-*myc* gene is highly elevated in most of the retinoblastomas examined. This finding suggests that N-*myc* gene may have a primary role in the tumorigenesis of retinoblastoma.

Genomic DNA was prepared from 10 primary retinoblastomas, 3 of which are described here for the first time. The other seven tumours have been karyotyped and reported previously[8] (Table 1). Various oncogenes were used as probes in the Southern genomic blotting analysis[15,16]. We found that two tumours (LA-RB70 and LA-RB63-1A), both of which contain extrachromosomal double minutes (DMs) (Table 1), and the Y79 cell line which contains a homogeneously staining region (HSR), all possessed a 2-kilobase (kb) *Eco*RI fragment in addition to the 13.5-kb c-*myc* fragment which demonstrates homology to the v-*myc* probe (data not shown). Both DMs and HSRs have been implicated in gene amplification of the N-*myc* in neuroblastoma[17,18]. Our results suggest that the retinoblastomas LA-RB70, LA-RB63-1A and Y79 show a similar amplification of the N-*myc* gene. Because the homology between c-*myc* and N-*myc* is limited[17,18], the N-*myc* sequences must be amplified to be detected by the v-*myc* probe in the Southern blotting. However, we found no gross abnormalities of the c-*myc* gene in these samples.

To confirm that the 2-kb *Eco*RI fragment is the N-*myc* gene, the pNb-1 plasmid (provided by Dr M. Bishop), which contains part of the known N-*myc* gene, was used as probe. We found that LA-RB70, LA-RB63-1A and the Y79 cell lines do indeed contain one amplified 2-kb *Eco*RI fragment which hybridizes with the pNb-1 probe (Fig. 1*a*). To exclude the possibility of technical artefacts, the pT24-C (c-H-*ras*) probe was also used in the same blot. Two *Eco*RI fragments of about 15 and 9 kb were hybridized with approximately equal intensity in every lane of DNA (data not shown). The extent of the amplification of the N-*myc* gene was determined by applying different amounts of DNA for Southern blotting. As Fig. 1*b* shows, compared with the level of N-*myc* gene in normal fibroblasts or other tumours, LA-RB70 and Y79 contain a 100–200-fold amplification of the N-*myc* gene and LA-RB63-1A contains a 10–20-fold amplification. Amplification of the N-*myc* gene in Y79 cells has also been described by Kohl *et al.*[18]. As the Y79 cell line has been in culture for many years, the amplification of the N-*myc* gene may be a result of clonal selection in culture rather than amplification in the original cells. However, the fact that the gene is amplified in the LA-RB70 and LA-RB63-1A tumours indicates that it can be amplified in the primary retinoblastoma or early passaged tumour cells.

Meanwhile, we prepared and analysed DNA from the fibroblasts of three patients with retinoblastomas—LA-RB63-1A (a patient whose tumour showed amplification of the N-*myc* gene), LA-RB69 and LA-RB73—as well as from fibroblasts of one normal human embryo. No amplification of the N-*myc* gene was found in these cells (Fig. 1*c*). These results indicate that as

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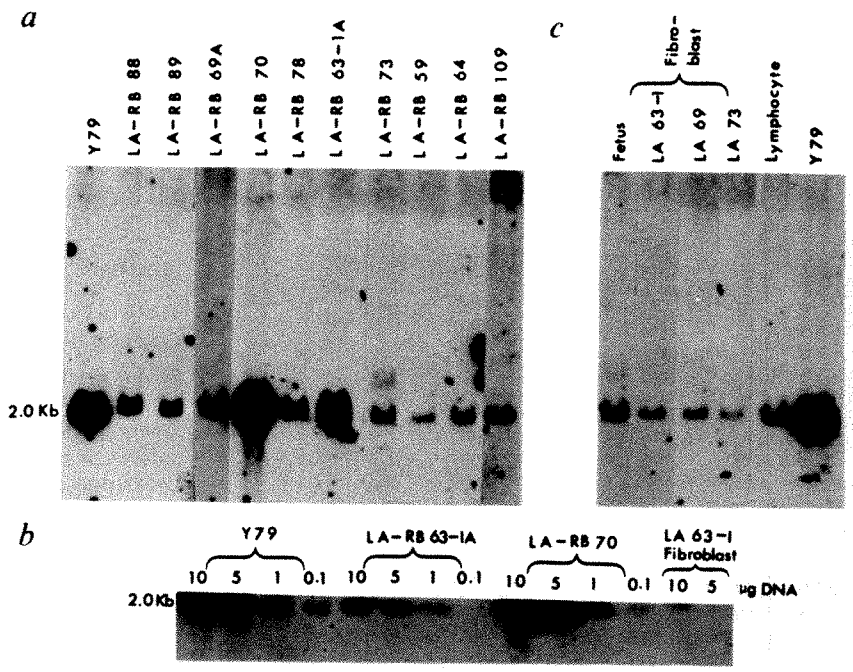


Fig. 1 Southern blot analysis of DNA from retinoblastomas and normal fibroblasts, probed with ^{32}P-labelled pNb-1 DNA. *a*, Amplification of N-*myc* gene in retinoblastoma DNA. A 2-kb *Eco*RI resistant fragment representing the N-*myc* gene was hybridized with pNb-1 probe in all DNA samples. Three cases, Y79, LA-RB70 and LA-RB63-1 contained an amplified N-*myc* gene. In addition to the 2-kb fragment, a 3-kb fragment of LA-RB73 was also hybridized. The significance of this hybridization is not known. *b*, Determination of the N-*myc* gene amplification fold. Different amounts of DNA were digested with *Eco*RI, and following Southern analysis, probed with pNb-1. Fibroblast DNA from the LA-RB63 patient was used as a standard for comparison. Part of the blot containing the 2-kb fragment region is shown in the figure. *c*, Normal fibroblast DNA from retinoblastoma patients had no amplification of the N-*myc* gene. Fibroblast DNA from three retinoblastoma patients, including one whose retinoblastoma contained N-*myc* amplification, one normal fetus and one adult lymphocyte DNA were subjected to Southern analysis, then probed with pNb-1 as described below. In the same blot, an equal amount of Y79 DNA was used for positive control.

Methods: Genomic DNA was prepared by the standard method[25] and ~10 µg of each DNA sample were digested by *Eco*RI to completion, electrophoresed through 0.8% agarose and blotted onto nitrocellulose. Hybridization with nick-translated ^{32}P-labelled pNb-1 DNA (specific activity 2.5×10^8 c.p.m. µg^{-1}) was in 40% formamide, 5×SSC at 42 °C for 36–48 h. Washing was done in 0.2×SSC, 0.5% SDS at 55 °C.

N-*myc* gene amplification occurred solely in tumour tissue, expression of this sequence could have an aetiological role in retinoblastoma formation.

It remained possible that abnormalities of other oncogenes existed in the eight tumours in which N-*myc* was not amplified at the DNA level. However, when *ras*, *ets*, *myb*, *erb* and *fps* were used as probes[10] to determine if there had been any rearrangements or other changes of these oncogenes, no gross abnormalities were found in any tumour (data not shown), although we cannot exclude the possibility that a point mutation has occurred in those genes which could be important for tumour formation.

Gene amplification is only one of several mechanisms for increasing gene expression[19]. To determine whether there was increased expression of N-*myc* in retinoblastoma at the mRNA level and whether this expression is specifically associated with gene amplification, we prepared RNA from 10 individual tumours, the Y79 cell line, four different fibroblast samples, one human fetal retina and one bovine retina. This RNA was then used to perform RNA dot analysis[20] using the pNb-1 as probe. We found that the Y79 cell line and four retinoblastomas (LA-RB63-1, LA-RB70, LA-RB78 and LA-RB109) expressed about 100-fold higher levels of N-*myc* mRNA than did retinal cells. The other tumours also had elevated levels of N-*myc* mRNA expression. However, N-*myc* expression was essentially undetectable in the fibroblasts (Fig. 2).

The LA-RB63-1A and LA-RB70 tumours as well as the Y79 cell line contained an amplified N-*myc* gene and consequently the presence of a high level of N-*myc* mRNA was anticipated. However, the other tumours did not contain detectable amplification of the N-*myc* gene, yet expressed a high level of N-*myc* mRNA, varying within four to fivefold. We do not know the significance of this difference. This increased expression might be due to the loss or inactivation of both alleles of a regulatory gene or a mutation at the regulatory site of the N-*myc* gene. If the first possibility is true, the *Rb* gene in 13q14 might actually be a regulator of N-*myc* expression.

We also analysed mRNA from neuroblastoma cell lines LA-N-1, LA-N-5 and SKN-SH as controls. The LA-N-1 and LA-N-5 cells showed a high level of N-*myc* expression[18] whereas two SKN-SH cell line variants contained neither amplified levels of the N-*myc* gene nor increased mRNA expression (Fig. 2). We emphasize that N-*myc* amplification appears to occur primarily

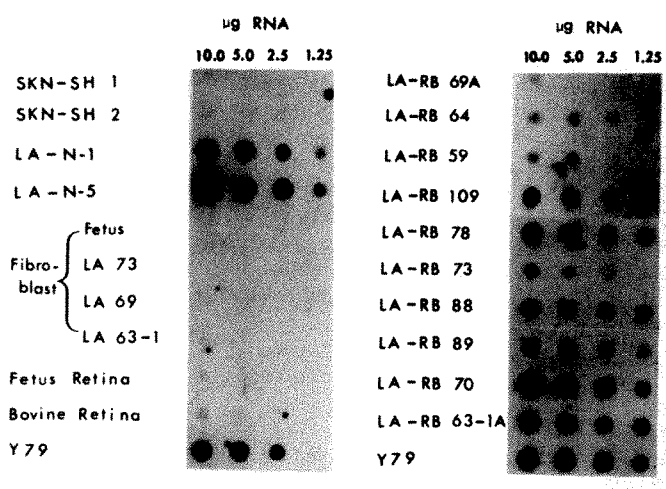


Fig. 2 Dot analysis of RNA from retinoblastoma, fibroblast, neuroblastoma and retina cells. Total cytoplasmic RNA was prepared by a method using Nonidet-P40 to lyse the cell membrane, followed by phenol–chloroform extraction[25]. RNA was precipitated by ethanol and quantitated with A_{260} and directly dotted onto a nitrocellulose membrane in 20× SSC. Hybridization was in 50% formamide and 5× SSC at 42°C with pNb-1 as probe. Washing was done in 0.2× SSC and 0.5% SDS at 60°C. Due to the limited amount of samples, some of the lanes contain RNA only in the first one or two dots.

in the advanced metastatic stages of neuroblastoma[21], which implies that the N-*myc* gene may be involved in the progression of malignancy in this disease. In contrast, except for LA-RB78 which was obtained from a rib metastasis, all other retinoblastomas with increased N-*myc* either at the gene or mRNA level were obtained as relatively small non-metastatic intraocular tumours. As these tumours included both hereditary (bilateral) and non-hereditary (most unilateral) cases (Table 1), the possibility exists that increased N-myc expression has a more fundamental role in retinoblastoma formation.

Although the N-*myc* gene has a limited sequence homology to the c-*myc* gene, the oncogenic potential of this gene is unknown. A suitable *in vitro* assay system to test the transforming ability of the N-*myc* gene should provide information on this issue. Nevertheless, the positive correlation between N-*myc*

Table 1 Summary of retinoblastomas used in the present study

Tumour designation	Tumour material used	Bilateral (B) or unilateral (U) case	Presence of DM or HSR	N-*myc* gene amplification	N-*myc* mRNA expression
LA-RB59	SQP 1*	U	–	—	+
LA-RB63-1A	SQP 1	B	+	10–20	+++
LA-RB64	SQP 1	U	–	—	+
LA-RB69A	EP 9†	B	–	—	+
LA-RB70	Primary	U	+	100–200	+++
LA-RB73	Primary	B	–	—	+
LA-RB78	SQP 2	U	–	—	+++
LA-RB88	Primary	B	–	—	++
LA-RB89	Primary	U	–	—	++
LA-RB109	Primary	U	–	—	+++
Y79	Cell line	B	+	100–200	+++

Tumours LA-RB59 to LA-RB78 have been described in ref. 8. Tumour LA-RB78 was obtained from rib metastasis rather than intraocular tumour. DM, extrachromosomal double minutes; HSR, homogeneously staining region; +, high level of expression; –, not found.

*Subcutaneous passage in nude mice.

†Eye passage in nude mice.

amplification in neuroblastoma and N-*myc* expression in the retinoblastoma strongly suggests that the N-*myc* gene may implies that the N-*myc* gene may be involved in the progression of malignancy in this disease. In contrast, except for LA-RB78 which was obtained from a rib metastasis, all other retinoblastomas with increased N-*myc* either at the gene or mRNA level were obtained as relatively small non-metastatic intraocular tumours. As these tumours included both hereditary (bilateral) and non-hereditary (most unilateral) cases (Table 1), the possibility exists that increased N-*myc* expression has a more fundamental role in retinoblastoma formation.

Several human tumours, including retinoblastoma, Wilms' tumour and neuroblastoma, are associated with specific chromosomal deletions[23]. The recessive nature of a relevant gene for tumour formation in the deleted band has been demonstrated for the *Rb* gene[1,3,4] and is likely for Wilms' tumour[24]. We speculate that recessive genes like the *Rb* gene in these tumours may have a regulatory role to modulate a set of proto-oncogenes. This hypothesis can be tested when the molecular nature of the *Rb* gene is elucidated.

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Identification of a form of the avian erythroblastosis virus *erb-B* gene product at the cell surface

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Avian erythroblastosis virus (AEV) induces both erythroblastosis and fibrosarcoma in chickens[1]. The viral oncogene responsible for these diseases, *erb*, is divided into two regions, *erb-A* and *erb-B*, although recent evidence suggests that it is primarily the *erb-B* gene product that is responsible for the transforming activity[2–5]. The *erb-B* gene product has been reported previously to be a membrane glycoprotein of 68,000 molecular weight (MW), gp68^{erb-B} (refs 6, 7). However, we show here that gp68^{erb-B} is an intracellular precursor which is modified further to a 74,000 MW protein, gp74^{erb-B}. By the criteria of resistance to digestion with endoglycosidase H, subcellular fractionation and inhibition of biosynthesis by the ionophore monensin, gp74^{erb-B} appears to be located at the cell surface. Recently, a comparison of the *erb-B* sequence[8] with that of the epidermal growth factor (EGF) receptor has shown that these two genes are highly homologous[9], and that *erb-B* appears to represent a truncated form of this growth factor. In light of these data the identification of gp74^{erb-B} at the plasma membrane suggests that this may be the functionally important form of the *erb-B* gene product.

Identification of homology between the *erb-B* gene product and the tyrosine kinase domains of other viral oncogenes[8] prompted us to propose that the *erb-B* gene product may function as a growth factor receptor[6,10,11]. Our hypothesis has recently been confirmed with the demonstration that *erb-B* shows near identity with the carboxy-terminal third of the human EGF receptor[9], and suggests that *erb-B* functions as a truncated growth factor receptor. However, this highlighted the major problem with our original hypothesis, namely the inability to detect a form of the *erb-B* glycoprotein that exhibited the hallmarks of a classic plasma membrane protein, for if *erb-B* functions as a growth factor receptor it should lie in the plasma membrane. It was therefore possible that a more mature form of the *erb-B* protein existed which we had failed to identify. We considered this highly possible, as our antisera raised in tumour-bearing rats recognized both the *erb-A* and *erb-B* proteins, and a form of the *erb-B* protein could be obscured by the *erb-A*-encoded protein p75^{erb-A} (ref. 12).

While screening antiserum from tumour-bearing rats which had been injected with lower numbers of AEV-transformed rat-1 cells than used previously[6], we identified 10 out of 60 animals tested which produced *erb-B*-specific antibodies. These antisera were used to analyse the *erb-B* proteins in greater detail. Immunoprecipitation analysis (Fig. 1*a*, track 2) revealed that these antisera precipitated only the *erb-B* proteins gp66 and gp68 from the AEV-transformed erythroblasts, whereas our original tumour-bearing antisera precipitated both the p75^{erb-A} and the *erb-B* proteins (Fig. 1*a*, track 3). Normal rat serum did not precipitate any of these proteins (Fig. 1*a*, track 4).

Our initial aim in preparing *erb-B*-specific antisera was to identify forms of the *erb-B* glycoprotein that had gone undetected in our previous studies. An examination of the ^{35}S-methionine-labelled proteins precipitated by the *erb-B*-specific serum failed to reveal any additional proteins (Fig. 1*a*, track 2). However, when the AEV-transformed erythroblasts were labelled for 16 h with ^{3}H-glucosamine, an additional protein of 74,000 MW, gp74, was obtained (Fig. 1*a*, track 1). This protein

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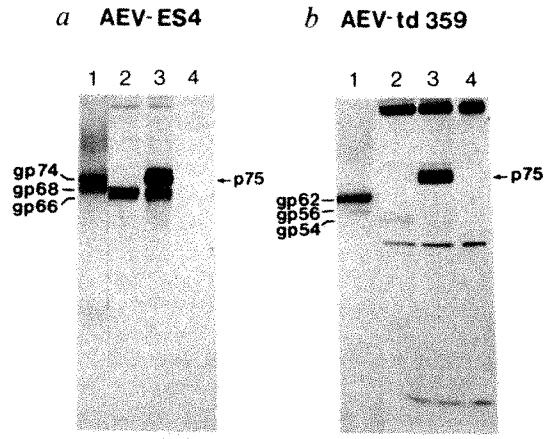


Fig. 1 Identification of *erb-B*-specific antiserum. Erythroblasts transformed by AEV-ES4 (panel *a*) or an isolate of AEV-*td*359 recovered from an infected chicken (panel *b*) were labelled with 100 μCi of ^{35}S-methionine for 30 min (tracks 2–4) or 500 μCi ^{3}H-glucosamine for 16 h (track 1). Immune precipitates were formed using rat anti-*erb-B* serum (tracks 1, 2), rat anti-*erb-A* plus *erb-B* (track 3) or normal rat serum (track 4). The precipitates were analysed by SDS-polyacrylamide gel electrophoresis and the labelled proteins were visualized by fluorography. The films were exposed for 2 days for tracks 2–4, and 3 weeks for track 1. Anti-*erb* serum was prepared by injection of 10^5–10^6 AT1a cells intraperitoneally into 10–14-day-old (Fischer × Wistar)F_1 rats as described previously[6]. The sera were collected 10–12 weeks later and tested for anti-*erb* activity by immunoprecipitation.

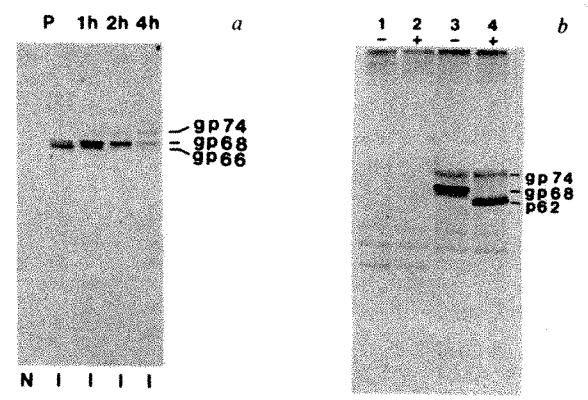


Fig. 2 Pulse-chase and endo-H digestion analysis of the *erb-B* glycoprotein. *a*, Erythroblasts transformed by AEV-ES4 were labelled for 30 min with 100 μCi ^{35}S-methionine. The cells were then pelleted and resuspended in complete growth medium. An aliquot of cells was removed for time point zero (P) and subsequent aliquots were removed after 1, 2 or 4 h. Detergent lysates were prepared and immune precipitates formed with either normal rat serum (N) or rat anti-*erb-B* serum (I). *b*, AEV-ES4-transformed erythroblasts were labelled for 30 min with ^{35}S-methionine, pelleted and resuspended in complete growth medium. The cells were then incubated for a further 3 h, collected and detergent lysates prepared. Immune precipitates were then formed with either normal rat serum (tracks 1, 2) or rat anti-*erb-B* serum (tracks 3, 4). The immune precipitates were then halved and one-half was treated with endo-H (+) as described previously[15]. The other half of the sample was treated in exactly the same way but without the addition of endo-H(−). All samples were analysed on a 10% SDS-polyacrylamide gel.

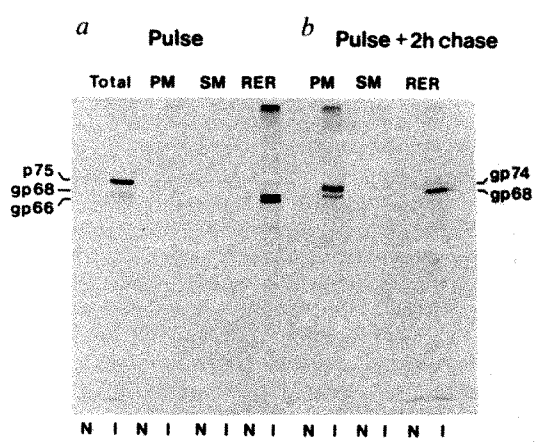


Fig. 3 Localization of gp74^{erb-B} to the plasma membrane. AEV-ES4-transformed erythroblasts were labelled for 30 min with 500 μCi of ^{35}S-methionine, and pelleted at 1,500*g* for 5 min. They were then resuspended in growth medium, and an aliquot (~10%) of the cells was taken to check the total incorporation (Total). From the remainder, half were removed for time point zero (*a*), the rest were incubated for a further 2 h (*b*). Both cell populations were then fractionated into plasma membrane (PM), smooth membrane (SM) and rough endoplasmic reticulum (RER)-enriched membrane populations, the fractions were characterized by buoyant density, RNA content and the presence of marker enzymes as described previously[6,16,17]. The membranes were dissolved in 0.5% NP40, 0.5% DOC, 50 mM Tris-HCl *p*H 8.1, 100 mM NaCl, and immune precipitates formed with either normal rat serum (N) or anti-*erb-A* + *B* serum (I). The precipitates were analysed on a 10% SDS-polyacrylamide gel and the proteins visualized by fluorography after 10 days' exposure.

was precipitated by the *erb-B*-specific serum from extracts of AEV-transformed erythroblasts (Fig. 1*a*, track 1) or fibroblasts (data not shown), suggesting that it was either an *erb-B*-encoded protein or a cellular protein. To distinguish between these possibilities, we used AEV-rtd359, a back mutant of AEV-*td*359 which has recovered the ability to transform erythroblasts but which has a deletion in *erb-B* and synthesizes *erb-B* proteins that are 12,000 daltons smaller than the wild-type protein (T. Graf, M.J.H., H.B. and B. Vennström, unpublished observations). Therefore, if gp74 was in fact encoded by *erb-B*, one would expect a glucosamine-labelled protein of a smaller size. The ^{35}S-methionine-labelled *erb-B* protein of AEV-rtd359 had MWs of 54,000 and 56,000 (Fig. 1*b*, track 2), the ^{3}H-glucosamine-labelled proteins were of MWs 56,000 and 62,000 and no gp74 was identified in these cells (Fig. 1*b*, track 1). These data show that the gp74 glycoprotein is not a cellular protein, as it would have been detected in all the transformed erythroblasts. In addition, because there is a ^{3}H-glucosamine-labelled protein 6,000–10,000 daltons larger than the largest ^{35}S-methionine-labelled *erb-B* protein in both AEV strains, the data strongly suggest that these larger forms are products of the *erb-B* gene.

The presence of ^{35}S-methionine-labelled gp74 was investigated by pulse-chase analysis and precipitation of the lysates with *erb-B*-specific antiserum. Following a 30-min pulse-label, both gp66 and gp68 were found in the cells (Fig. 2*a*), and during the chase period gp66 was processed into gp68, as shown previously[6]. However, after a 1-h chase period, gp74 was also found in the cells, the amount increasing after 2 h and reaching a steady-state level by 4 h (Fig. 2*a*). Interestingly, the amount of label found in gp74 accounts for only ~25% of that initially detected in gp66 and gp68 (Fig. 2*a*). This means either that only 25% of the newly synthesized *erb-B* protein was processed into the gp74 form and the rest was rapidly degraded intracellularly or that the gp74 protein is being sequestered to a pool that is no longer detectable.

Digestion with the enzyme endoglycosidase H (endo-H)[13] had shown previously that both gp66 and gp68 were sensitive to digestion by this enzyme[6]. To see if gp74 was also sensitive to digestion, cells were labelled for 30 min with ^{35}S-methionine and chased for 3 h to allow the formation of gp74. Figure 2*b* shows that the gp68 form is still sensitive to endo-H, whereas the gp74 form is completely resistant to this enzyme, indicating that the carbohydrate chains on gp74^{erb-B} have been processed into the complex form.

Analysis of gp66^{erb-B} and gp68^{erb-B} by subcellular fractionation had localized these proteins to the microsomal fraction of the cell[6]. However, these previous studies had not distinguished

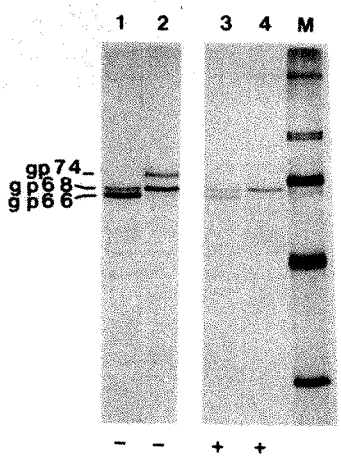


Fig. 4 Monensin inhibits the formation of gp74$^{erb\text{-}B}$. AEV-transformed erythroblasts were preincubated for 2 h in the absence (−) or presence (+) of 10 μM monensin (a kind gift of Dr M. Owen). Cells were then labelled for 30 min with ^{35}S-methionine, one population in the absence and the other in the presence of 10 μM monensin. The cells were pelleted and half taken from each population for the pulse time point (tracks 1, 3), the rest being resuspended in growth medium and incubated for a further 2 h, again in the absence (−) or presence (+) of 10 μM monensin (tracks 2, 4). Detergent extracts were prepared and immune precipitates formed with anti-*erb-B* serum. The precipitates were analysed on a 10% SDS-polyacrylamide gel, and the proteins visualized by fluorography. Exposure time 5 days. M denotes ^{14}C-labelled molecular weight standards from Amersham of 200,000, 94,000, 68,000, 45,000 and 30,000.

in which membrane populations the proteins resided, nor had any biosynthetic pathway for the processing of the *erb-B* glycoprotein been elucidated. We therefore extended this analysis by subfractionating the microsomes into rough endoplasmic reticulum, a smooth membrane fraction and plasma membranes. The localization of the *erb-B* proteins was also analysed following a pulse-label of 30 min and a chase of 2 h to monitor any redistribution of the various glycoproteins (Fig. 3). Initially, both gp66$^{erb\text{-}B}$ and gp68$^{erb\text{-}B}$ were located in the rough endoplasmic reticulum, as would be expected for intrinsic membrane glycoproteins (Fig. 3*a*). After 2 h, gp74$^{erb\text{-}B}$ was detected, but it was located exclusively in the plasma membrane-enriched fraction of the cell (Fig. 3*b*). In contrast, gp68$^{erb\text{-}B}$ was still mainly located in the rough endoplasmic reticulum fractions although a minor portion was also found in the plasma membrane-enriched fraction (Fig. 3*b*).

Monensin is a sodium ionophore which alters the intracellular transport of membrane glycoproteins[14]. Because gp74 seems to be the plasma membrane form of the *erb-B* gene, it was of interest to test whether monensin would inhibit its processing. A pulse-chase analysis performed in the presence and absence of 10 μM monensin revealed that in the absence of the ionophore the *erb-B* glycoproteins were processed to the gp74 form as usual (Fig. 4, tracks 1, 2). In contrast, although gp66$^{erb\text{-}B}$ was processed to gp68$^{erb\text{-}B}$ in the presence of monensin, no gp74$^{erb\text{-}B}$ was produced (Fig. 4, track 4). Therefore, monensin completely inhibits the processing of gp68$^{erb\text{-}B}$ to gp74$^{erb\text{-}B}$.

The production of antisera specific for the *erb-B* product allowed us to identify and characterize a previously unrecognized form of the *erb-B* glycoprotein, namely gp74$^{erb\text{-}B}$. Moreover, the observation that gp68$^{erb\text{-}B}$ is sensitive to endo-H whereas gp74$^{erb\text{-}B}$ is not, implies that the processing of gp66$^{erb\text{-}B}$ to gp68$^{erb\text{-}B}$ takes place in the rough endoplasmic reticulum whereas the processing of gp68$^{erb\text{-}B}$ to gp74$^{erb\text{-}B}$ probably occurs in some compartment of the Golgi complex. The sensitivity of the processing of gp68$^{erb\text{-}B}$ to gp74$^{erb\text{-}B}$ to the presence of 10 μM monensin, an ionophore which interferes with the passage of certain glycoproteins through the Golgi apparatus[14], is consistent with this hypothesis. Taken together with the cell fractionation studies, these data imply that gp74$^{erb\text{-}B}$ is the plasma membrane form of the *erb-B* gene product; a similar molecule has recently been identified by M. L. Privalsky and J. M. Bishop (personal communication).

With regard to the finding that the *erb-B* gene appears to be a truncated form of the growth factor receptor, most of the coding information removed comes from the amino-terminal end of the EGF receptor[9], which would include the EGF binding site. This helps to explain two recent observations. First, although there is near identity at the amino acid level between *erb-B* and EGF receptor, antisera against these two proteins do not cross-react (M.J.H., J. Downward and M. Waterfield, unpublished observations). However, this is not surprising as the anti-EGF receptor antibodies are directed against its amino terminus, which is missing from *erb-B*, and antibodies against the external domain of *erb-B* probably cannot gain access to this region in the EGF receptor because in *erb-B* the domain has a MW of only ~12,000 compared with 115,000 for the EGF receptor. Second, no kinase activity associated with *erb-B* has been detected in the standard immune complex assay[10]. This is consistent with its having lost the binding site for the growth factor, interaction with which would presumably stimulate activity. However, this does not mean that *erb-B* could not function as a kinase in certain specific conditions, and this enzymatic function may yet be involved in *erb-B*-induced cell transformation.

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Molecular cloning of the whole biosynthetic pathway of a *Streptomyces* antibiotic and its expression in a heterologous host

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The application of molecular cloning to antibiotic-producing microorganisms should lead to enhanced antibiotic productivity and to the biosynthesis of novel antibiotics by *in vitro* interspecific recombination[1,2]. To allow such approaches, the genes for antibiotic synthesis must be isolated, analysed and perhaps modified. Certain *Streptomyces* species produce nearly two-thirds of the known natural antibiotics[3]; the recent development of cloning systems in the genus[4–7] makes it possible to isolate and analyse *Streptomyces* genes. However, antibiotics are metabolites which require sets of several enzymes for their synthesis and attempts to isolate the corresponding genes have so far yielded clones carrying either individual genes of the set, or only incomplete gene sets[8–11]. We describe here the isolation of a large continuous segment of *Streptomyces coelicolor* DNA which apparently carries

the complete genetic information required for synthesis of an antibiotic, actinorhodin, from simple primary metabolites. Not only can the cloned DNA 'complement' all available classes of actinorhodin non-producing mutants of *S. coelicolor* but, on introduction into a different host, *Streptomyces parvulus*, it directs the synthesis of the antibiotic. The tendency for the genes for antibiotic synthesis to be clustered together on the chromosomes of *Streptomyces* species[12] and the availability of plasmid vectors which can carry stable inserts of DNA larger than 30 kilobase pairs (kb) and which can be introduced efficiently into *Streptomyces* protoplasts, suggest that the experiments described have general significance for this area of biotechnology.

Actinorhodin[13] belongs to a class of antibiotics named isochromanequinones which also includes granaticin[14], kalafungin[15] and the nanaomycins[16]. It is derived biosynthetically from acetate units (acetyl coenzyme A) through the polyketide pathway[17]. Rudd and Hopwood[18] isolated 76 actinorhodin non-producing mutants (*act*) and classified them into seven phenotypic classes, where each class represented lesions in a different biosynthetic step. Mutants in six of the classes co-synthesized actinorhodin in combination with other mutants, allowing a tentative biosynthetic sequence to be deduced, as follows:

I,III → VII → IV → VI → V → actinorhodin.

Mutants of class II failed to co-synthesize actinorhodin in any combination and perhaps included polar and regulatory mutations. At least one mutation of each class was mapped to the same segment of the *S. coelicolor* chromosome[18]. The actinorhodin system therefore offered the possibility of cloning a DNA fragment encoding a complete biosynthetic pathway provided that a suitable vector was available, with the eventual aim of analysing its physical organization and regulation.

Reconstruction experiments indicated that Act$^+$ colonies could be detected easily among a large number of *act* colonies by the formation of the blue diffusible actinorhodin when grown on R2YE medium[6]. 'Complementation' of an *act* mutant, detected by restoration of the pigmented phenotype, was therefore used as the screen for desired clones. A mutant of *act* class V, blocked in a late step of the biosynthesis (and also carrying a *red* mutation to abolish production of the other pigment, undecylprodigiosin[19]) was used as the recipient for cloned DNA from the wild-type *act*$^+$ strain.

The plasmid SCP2*, a 31-kb sex factor of *S. coelicolor*[20,21], has recently been developed into a series of cloning vectors by deletion of dispensable DNA and by addition of drug resistance markers[22]. One such vector is pIJ922 (25 kb) which contains a gene for thiostrepton resistance (*tsr*) from *Streptomyces azureus*[6] and has single sites for several restriction enzymes, including *Bam*HI, within a non-essential region. DNA of pIJ922, purified by CsCl–ethidium bromide density gradient centrifugation[21], after alkaline lysis[23], was digested with *Bam*HI. After phenol extraction, 0.5 μg of the linearized plasmid was mixed with 5 μg of chromosomal DNA from an *act*$^+$ *S. coelicolor* strain which had been partially digested with *Mbo*I and size-fractionated in a sucrose density gradient[24] to give fragments in the size range 15–30 kb. The DNA mixture, after ethanol precipitation, was dissolved in T4–DNA ligase buffer and ligated[25] at a final DNA concentration of 50 μg ml^{-1}. The ligated DNA was re-precipitated and dissolved in 25 μl TE (10 mM Tris-HCl, 1 mM EDTA, *p*H 8). An aliquot containing 4.4 μg of ligated DNA was used to transform[25] protoplasts of *S. coelicolor* SCP2$^-$ strain JF4 (*proA1 argA1 cysD18 strA1 act109 (V) redE60*)[8]. After allowing 20 h for phenotypic expression of thiostrepton resistance, the R2YE regeneration medium plates were overlaid with soft nutrient agar (Difco) containing thiostrepton to give a final concentration in the entire plates of 50 μg ml^{-1}.

Two blue colonies (presumptive clones) were detected among a total of ~8,000 transformants growing on 20 plates. Isolation of CCC DNA from the colonies revealed recombinant plasmids (named pIJ2300 and pIJ2301); each was introduced by transformation into *act* mutants of each of the seven classes, with the results shown in Table 1. pIJ2300 complemented all classes except VI, while pIJ2301 complemented classes V, VI and VII. Restriction maps of the plasmids revealed inserts of 34 and 16 kb in pIJ2300 and pIJ2301 respectively, with an apparently overlapping region of 12 kb (Fig. 1).

The entire insert of pIJ2300 was bounded by *Bam*HI sites. Moreover, preliminary subcloning experiments showed that class VI mutations could be complemented by a 4.5-kb *Bam*HI fragment uniquely present at the left-hand end of pIJ2301 (Fig. 1) and presumably the most distal region carrying biosynthetic genes. This suggested a strategy for constructing a single clone able to complement all available *act* mutants, and perhaps containing the entire chromosomal region responsible for actinorhodin biosynthesis. A mixture of pIJ2300 partially digested with *Bam*HI and pIJ2301 completely digested with *Bam*HI (plus *Eco*RI to prevent re-formation of pIJ2301) was ligated and used to transform protoplasts of strain JF3 (*act* class

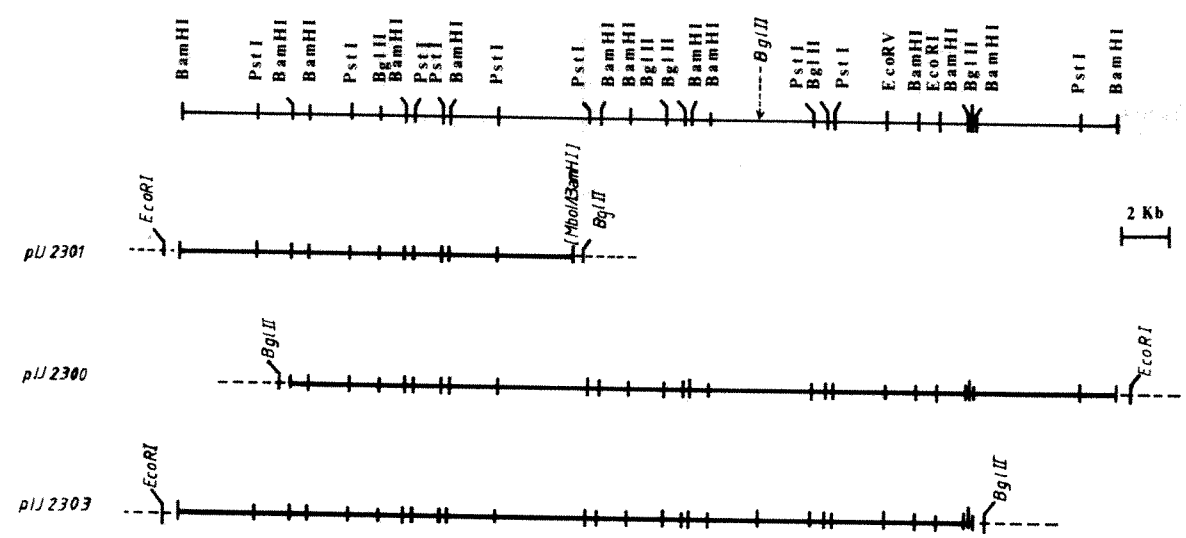


Fig. 1 Comparative restriction maps of *act*$^+$ clones pIJ2301, pIJ2300 and pIJ2303. The maps of pIJ2300 and pIJ2303 were derived from the maps of overlapping fragments subcloned into SCP2*-derived vectors. In addition to loss of a 6-kb *Bam*HI fragment from the right-hand end of pIJ2300, pIJ2303 carries a new *Bgl*II site (arrow). From the results of subcloning experiments, this discrepancy does not affect the ability of pIJ2303 to 'complement' all of the *act* mutants tested, and may be explicable by homologous recombination between pIJ2300 (with wild-type DNA) and the chromosome of the *act* mutant in *S. coelicolor* JF4, giving rise to a heterogeneous plasmid population. Dashed lines indicate pIJ922 vector DNA, with the *Eco*RI and *Bgl*II sites shown for orientation.

Table 1 Complementation test of *act*$^+$ recombinant plasmids with *act* *S. coelicolor* mutants and expression of the clones in *S. parvulus*

Recipient strain		Blue pigment produced by strains carrying		
John Innes stock no.	*act* class	pIJ2300	pIJ2301	pIJ2303
*S. coelicolor**				
JF1	II	+	−	+
TK17	I	+	−	+
TK18	III	+	−	+
B257	VII	+	+	+
TK16	IV	+	−	+
JF3	VI	−	+	+
JF4	V	+	+	+
S. parvulus 2283†		−	−	+
S. parvulus 2618‡		−	−	+

* All the strains are mutational and recombinational derivatives of *S. coelicolor* A3(2); their complete genotypes are as previously reported[8].
† Chromosomal marker: *str-1*.
‡ Chromosomal markers: *cys-1 his-5*.

VI). Of 20 blue transformants, one carried a plasmid (pIJ2303) that complemented strongly *act* mutants of all seven classes. The restriction map of pIJ2303 (Fig. 1) demonstrated insertion of the required 4.5-kb *Bam*HI fragment from pIJ2301 at the left end of the pIJ2300 insert and in the original orientation of pIJ2301. At the opposite end, pIJ2303 showed deletion of a 6-kb *Bam*HI fragment present in pIJ2300, but evidently this was not required for complementation of any of the available *act* mutants.

To determine whether pIJ2303 carried the entire set of *act* biosynthesis genes, pIJ2300, pIJ2301 and pIJ2303 were introduced by transformation into genetically marked strains of *S. parvulus* ATCC 12434. This strain does not produce actinorhodin and is not a close relative of *S. coelicolor* A3(2) as judged either by conventional taxonomy or by DNA–DNA hybridization[26]. It is, however, a host for SCP2 plasmids. Only those transformants that grew on R2YE plates containing thiostrepton and which carried pIJ2303 were blue (Table 1). Thin-layer chromatography of methanol extracts[19] of the *S. parvulus* host cultures and the same strains containing each of the three recombinant plasmids showed the presence of a blue pigment only in clones carrying pIJ2303. This blue pigment co-migrated with a corresponding pigment in extracts from *act*$^+$ but not *act* *S. coelicolor* cultures and had acid–base indicator properties characteristic of actinorhodin. The amounts of the blue pigment in extracts of *S. parvulus* carrying pIJ2303 were similar to those of wild-type *S. coelicolor*. This result suggests that the entire DNA sequence corresponding to the structural genes for actinorhodin biosynthesis is carried by pIJ2303.

The success of the cloning experiments reported here may well have depended on the use of a low-copy number vector (the estimated copy number of the parent SCP2 is one per chromosome of *S. coelicolor*[21]) so that the recipient strain is not likely to be placed at a disadvantage by possible high-level expression of physiologically active gene products. This view is supported, though not proved, by our failure to isolate any Act$^+$ clones in extensive trials with the high-copy number vector pIJ702 (ref. 27) despite the fact that this vector has proved convenient for the cloning of comparatively short segments of DNA carrying other genes[8,28]. Vectors such as pIJ922, and other SCP2* derivatives recently developed by Lydiate[22], appear to be particularly suitable for the stable maintenance of large fragments of cloned DNA. Moreover, the large recombinant plasmids are readily isolated from *Streptomyces* cultures[23], and transform *Streptomyces* protoplasts efficiently. Thus pIJ2300, of 59 kb, yielded 10^6 transformants per μg of DNA in optimal conditions, compared with 10^7 for the pIJ922 vector.

As the entire carbon skeleton of actinorhodin is derived from acetate[17], no specialized primary metabolites are required for its biosynthesis, a factor which may have contributed to the efficient expression of pIJ2303 in *S. parvulus*, leading to production of actinorhodin. Many important antibiotics are also polyketides—including the macrolides, tetracyclines and rifamycins. It is generally accepted that the primary carbon skeletons of polyketides are assembled on multienzyme complexes (or multifunctional enzymes) analogous to fatty acid synthetases[29], and the genes or gene clusters that encode polyketide synthetases may have had a common evolutionary origin, perhaps shared with fatty acid synthetases. Thus, the availability of clones carrying the presumptive actinorhodin 'synthetase' may be useful, not only as a model system, but possibly as DNA probes for the isolation of other antibiotic synthetases.

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Ha-*ras* oncogenes are activated by somatic alterations in human urinary tract tumours

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DNA-mediated gene transfer (transfection) studies using NIH 3T3 cells as recipients have demonstrated the presence of transforming genes (oncogenes) in diverse human tumours (for reviews see refs 1, 2). A large proportion of oncogenes so far detected by DNA transfection are related to the Ha-*ras onc* gene of Harvey (and BALB) murine sarcoma viruses (MSV)[3–6], Ki-*ras*, the oncogene of Kirsten MSV[3,7,8], and a third member of the *ras* gene family, N-*ras* (refs 8–12). Individual tumours of many different organs

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have been associated with the activation of members of the *ras* gene family. We now present the first systematic survey of human urinary tract tumours processed immediately after surgery, as well as normal tissues from the same patients, to detect the presence of such genes. We demonstrate activation of Ha-*ras* as an oncogene in around 10% of randomly selected urinary tract tumours as well as direct evidence that oncogene activation is the result of a somatic event which is selected for within the tumour cell population.

Urinary tract carcinoma, at any site from renal pelvis to urethra, is considered to be a gross manifestation of a generalized neoplastic change of its epithelium, the urothelium. Multiple bladder carcinomas are not uncommon at cystoscopy and detailed histological examination of surgical specimens from patients with carcinomas of the bladder, renal pelvis and ureter has revealed a high incidence of neoplastic and preneoplastic lesions at sites other than the actual tumour[13,14]. To survey for oncogenes detectable by transfection analysis, high molecular weight DNA in sufficient quantity for analysis was obtained from 23 out of 32 randomly selected consecutive specimens of urinary tract tumours. The tumours were transitional cell carcinomas except for one adenocarcinoma.

Transforming activity was observed with two tumours. These two patients (JPT26 and JBT44) had no special features in terms of occupation, past history, or clinical or pathological characteristics of the tumour[15]. Moreover, there was no apparent correlation between the presence of an active oncogene and the degree of invasiveness of the tumours analysed. In each case, transforming activity survived a second cycle of transfection using high molecular weight DNAs of individual first-cycle NIH 3T3 transfectants (Table 1). Southern blotting analysis showed the presence of human repetitive sequences in all first- and second-cycle transfectants, implying that the foci observed were induced by human DNA (data not shown).

DNAs from JPT26- and JBT44-induced foci were hybridized with nick-translated probes specific to each of the *ras* oncogenes. Neither human Ki-*ras* nor N-*ras* probes detected DNA fragments other than the endogenous bands (data not shown). However, when *Bam*HI-digested transfectant DNAs were analysed for Ha-*ras*-related sequences, all tested JPT26 and JBT44 transfectants exhibited an additional 7-kilobase pair (kbp) DNA fragment (Fig. 1*a*). These findings identified the oncogene in each tumour as an activated allele of the Ha-*ras* proto-oncogene.

Accumulating evidence indicates that the major sites for the genetic lesions responsible for activation of *ras* oncogenes in human tumours are localized to positions 12 and 61 in their coding sequences[6,16–21]. An alteration at position 12 in human Ha-*ras* leads to loss of a restriction site for *Hpa*II–*Msp*I digestion, thus providing a means of molecular diagnosis of lesions at this position[22]. As Fig. 1*b* shows, DNA from JPT26 and JBT44 transfectants demonstrated a 355-base pair (bp) band (lanes 1 and 2, respectively) like that observed with human placenta DNA (lane 3), while the T24 transfectant DNA yielded the expected 411-bp band (lane 4). These results indicated that neither the JPT26 nor JBT44 oncogenes had undergone mutations affecting the 12th amino acid of its p21 coding sequence.

The recognition sequence for *Bst*NI is $CC^{A}_{T}GG$, whereas the normal sequence encompassing position 61 in the Ha-*ras* proto-oncogene is CCAGG (Fig. 2)[23,24]. Thus, any alteration in the sequence other than A to T would be expected to eliminate this *Bst*NI recognition site. As Fig. 2 shows, the electrophoretic mobilities of two *Bst*NI-digested DNA fragments hybridizing to the Ha-*ras* *Kpn*I–*Nco*I probe in a representative JBT44 transfectant (lane 4) were not distinguishable from those of a T24 transfectant, possessing a position 12 lesion (lane 1)[16–18]. The 158-bp band corresponded to the larger fragment observed with an intact *Bst*NI site at position 60–62. A transfectant containing the Hs242 oncogene, whose corresponding sequence at position 61, CCTGG (ref. 6), would also be recognized by *Bst*NI, demonstrated an identical pattern of DNA fragments (Fig. 2, lane 2). In contrast, a JPT26 transfectant DNA exhibited a different hybridizing DNA fragment, whose 173-bp size corresponded to that expected in the absence of a *Bst*NI cleavage site at position 60–62 (lane 3). Thus, these results established that the oncogene from patient JPT26 had suffered a genetic lesion affecting codons 60, 61 or 62.

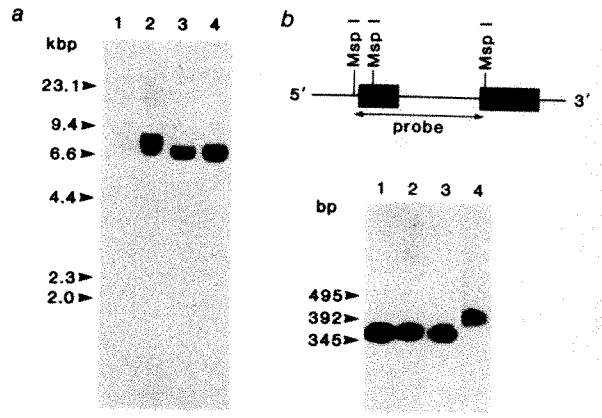


Fig. 1 Detection and identification of the human urothelial carcinoma transforming genes and their *Msp*I sensitivity at codon 12. 20 μg of high molecular weight DNA were digested with *Bam*HI (column *a*) or with *Msp*I and *Hpa*II (column *b*), electrophoresed in 1.2% or 2.0% agarose gel, respectively, transferred to nitrocellulose paper and hybridized as described previously[6,25]. *a*, DNAs from NIH 3T3 cells (lane 1), human placenta (lane 2), as well as representative first-cycle transfectants of JPT26 (lane 3) and JBT44 (lane 4) tumours were hybridized with the 6.6-kbp *Bam*HI fragment of the T24 oncogene, p24-c3 (ref. 17). In these conditions, the mouse endogenous 3.5-kbp Ha-*ras*-related fragment was not observed. Co-electrophoresed DNA fragments of *Hin*dIII-digested λc1857 DNA served as molecular weight standards. *b*, The upper panel shows the *Msp*I restriction map of the human Ha-*ras* proto-oncogene. The two solid boxes indicate the first and second exons from the 5′ to 3′ direction. In the lower panel, DNAs from representative first-cycle transfectants of JPT26 (lane 1), JBT44 (lane 2) and T24 (lane 4) tumours, as well as human placenta DNA (lane 3) were hybridized with the T24 *Msp*I fragment shown by the arrow in the upper panel. Co-electrophoresed DNA fragments of *Hin*cII-digested ΦX174RF DNA served as molecular weight standards. The 56-bp fragment was not apparent in these experimental conditions.

Table 1 Transforming activity of DNA from human urinary tract tumours

Donor DNA	Total no. foci/total DNA tested (μg) Primary	Secondary
T24 bladder carcinoma	85/400	—
JPT26 renal pelvic carcinoma*	8/320	37/240
JBT44 bladder carcinoma†	15/320	28/240

Human tumours were minced with scalpels within 4 h of surgical removal, incubated for 3 h at 37 °C with 200 μg ml^{-1} of proteinase K in the presence of 0.5% SDS and 10 mM EDTA, extracted with phenol and precipitated with ethanol. High molecular weight DNA was further purified by treatment with RNase, proteinase K and extracted with phenol/chloroform. These DNAs, as well as DNAs from representative first-cycle NIH 3T3 transfectants, were used to transfect onto NIH 3T3 cells by the calcium phosphate precipitation technique, as described previously[6]. Foci of transformed cells were scored at 21 days. Twenty-three tumours, including four non-invasive tumours, were tested. Two of these were cytological grade 1, 11 were grade 2 and 10 were grade 3 (ref. 15). In 10 cases, bladder tumours were multiple. Eight patients had a previous history of bladder tumours.

* JPT26, a 33-yr-old male who had the right kidney and ureter removed for a papillary non-invasive transitional cell carcinoma, grade 2 of the renal pelvis.

† JBT44, a 44-yr-old male who underwent transurethral resection of recurrent multiple transitional cell carcinomas of the bladder which were grade 2 and invaded the submucosa (P1).

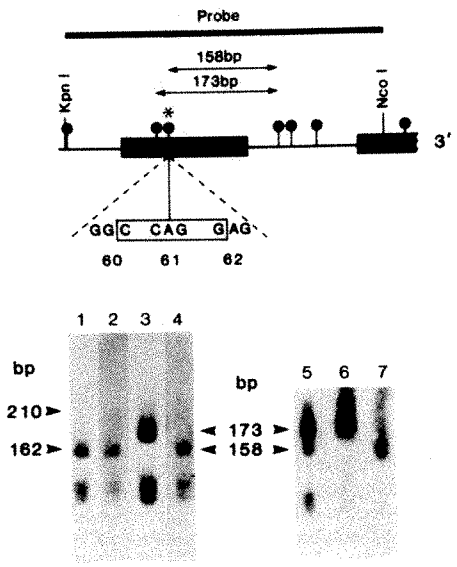


Fig. 2 *Bst*NI sensitivity encompassing codon 61 of the human Ha-*ras* gene in NIH 3T3 transfectants, JPT26 tumour and JPT26 normal DNAs. The upper panel shows the *Bst*NI restriction map of the human Ha-*ras* proto-oncogene. The two solid boxes represent the second and third exons from the 5′ to 3′ direction. ●, *Bst*NI cleavage sites: *, the *Bst*NI site encompassing codon 61. The nucleotide sequence surrounding this site is also shown. The numbers below the sequence indicate the amino acid codon number. The lower panel shows Southern blotting analysis of DNAs from representative NIH 3T3 transfectants of T24 (lane 1), Hs242 (lane 2), JPT26 (lanes 3, 6) and JBT44 (lane 4) tumours as well as JPT26 primary tumour (lane 5) and JPT26 normal peripheral leukocytes (lane 7). DNAs were digested with the restriction endonuclease *Bst*NI, electrophoresed in a 4% agarose–50% formamide gel[26], blotted to nitrocellulose paper and hybridized with the *Kpn*I–*Nco*I fragment of the human Ha-*ras* proto-oncogene as shown in the upper panel. Twice as much DNA (100 μg) was used in lane 5 as in the other lanes. The variable detection of the 128/123-bp DNA fragment (compare lanes 3 and 6) with the same JPT26 transfectant DNA reflects variability in the efficiency of transfer of low molecular weight DNA species to the nitrocellulose paper. Co-electrophoresed DNA fragments of *Hinc*II-digested ΦX174RF DNA served as molecular weight standards.

The availability of normal peripheral blood leukocytes from patients JPT26 and JBT44 made it possible to assess whether normal tissues from these patients contained normal or activated Ha-*ras* alleles. DNAs from these normal cells were assayed for transforming activity in the NIH 3T3 transfection assay. No focus-forming activity was detectable with either DNA, in conditions in which both tumour cell DNAs readily registered as positive (data not shown). When DNAs from the JPT26 primary tumour and normal peripheral blood leukocytes were analysed by a molecular genetic approach (Fig. 2), the Ha-*ras* *Kpn*I–*Nco*I probe hybridized to *Bst*NI-digested DNA fragments of 173 and 158 bp as well as to the 128/123-bp species in DNA of the primary JPT26 tumour (Fig. 2, lane 5). In contrast, normal peripheral blood leukocytes from patient JPT26 (lane 7) demonstrated only the 158-bp hybridizing DNA fragment. The 173-bp fragment specific for the altered Ha-*ras* allele present in the JPT26 transfectant (lanes 3, 6) was not detected in the normal cells. These results established that the altered Ha-*ras* allele was present in the JPT26 tumour but not in normal tissues from the same patient.

Transforming genes were detected by transfection analysis in 2 of 23 randomly selected primary human tumours of the urothelium. In both cases, Ha-*ras* oncogenes were identified as the source of transforming activity. Neither oncogene was activated by a lesion at position 12 in the coding sequence, consistent with other studies indicating that position 12 lesions in Ha-*ras* are not commonly detected in primary human tumours[22]. We demonstrated a genetic alteration(s) within codon(s) 60, 61 or 62 of the Ha-*ras* coding sequence of the JPT26 tumour by a *Bst*NI restriction site polymorphism. Since position 61 is a major 'hot spot' for activation of *ras* oncogene in human tumours[6,21], it is likely that the genetic alteration detected at this position of the JPT26 oncogene coding sequence is responsible for its activation as a transforming gene.

It is of critical importance to establish the direct relevance of an oncogene detected in a human tumour to the neoplastic process affecting that tumour cell. In the present studies, normal cell DNA from neither of the patients whose tumours contained Ha-*ras* oncogenes possessed a transforming gene detectable by transfection analysis. By restriction enzyme analysis, it was possible to obtain direct biochemical evidence that the genetic lesion in the JPT26 oncogene was not present in normal cellular DNA of this same patient. These findings establish that this genetic alteration is the result of a somatic event which must be powerfully selected for within the tumour and, thus, likely to contribute to its development.

Our results also suggest that that Ha-*ras* oncogenes are more commonly activated in urinary tract tumours than are N- or Ki-*ras* genes. This is in contrast to haematopoietic tumours, where N-*ras* appears to be the most frequently activated *ras* oncogene[8,12], or lung and colon carcinoma where the Ki-*ras* oncogene appears to predominate[3,7,19,20]. The frequency at which oncogenes were detected is likely to be an underestimate due to the inherent insensitivity of the transfection assay. Single base changes at either position 12 or 61 in the Ha-*ras* coding sequence will lead to loss of *Msp*I or *Bst*NI sites for 6/6 and 7/8 possible amino acid substitutions, respectively. Thus, a systematic survey of urinary tract carcinomas for these point mutations as well as for amplification or other rearrangements using biochemical approaches may provide a more accurate estimate of the frequency at which Ha-*ras* is activated as an oncogene in these naturally occurring human malignancies.

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Evolution of phosphofructokinase—gene duplication and creation of new effector sites

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Phosphofructokinases (PFK; EC 2.7.1.11) are tetrameric enzymes that have a key role in the regulation of glycolysis[1]; as such, they are subject to allosteric activation and inhibition by various metabolites[2]. Eukaryotic PFKs are about twice the size of prokaryotic enzymes and are regulated by a wider repertoire of effectors: for example, the subunit molecular weights of rabbit muscle (RM) PFK and *Bacillus stearothermophilus* (Bs) PFK are 82,000 and 36,000, respectively. Both enzymes are activated by ADP (or AMP), but RM-PFK is also activated by fructose bisphosphates (FBP) and inhibited by ATP and citrate. This, together with other evidence, has led to speculation that mammalian PFKs have evolved by duplication of a prokaryotic gene[3–5], although previous peptide analysis[6] failed to reveal internal homology in RM-PFK. Here we demonstrate clear homology among the N- and C-halves of RM-PFK and Bs-PFK, thus establishing an evolutionary relationship by series gene duplication and divergence. Furthermore, detailed knowledge of the Bs-PFK structure provides the basis for inferences concerning the structural organization of RM-PFK and the evolution of new effector sites in the enzyme tetramer.

The nearly complete amino acid sequence of RM-PFK is given in Fig. 1, together with that of Bs-PFK[7]. Strong homology is observed among the N- and C-halves of the RM-PFK molecule and Bs-PFK. The average minimum base differences per codon[8], calculated between the halves of the RM-PFK subunit and between each half of RM-PFK and the bacterial PFK are as follows: Bs-PFK versus N-half of RM-PFK, 0.82 (319 residues compared); Bs-PFK versus C-half of RM-PFK, 0.93 (289 residues compared); and N-half versus C-half, 1.02 (329 residues compared). The respective identities for each comparison are 44%, 34% and 32%. These numbers substantiate a significant degree of homology among all three sequences; the N-half of RM-PFK is especially homologous to the bacterial enzyme. Insertions in the RM-PFK halves are easily accommodated at the surface[9,10], and need not alter the basic architectural theme of Bs-PFK. Clearly, there is extensive internal homology in the RM-PFK subunit, and the mammalian enzyme arose by series gene duplication of an ancestral message related to that of Bs-PFK.

The evolution of proteins proceeds with conservation of three-dimensional structure, despite wide-ranging diversification of amino acid sequence[11,12]. Therefore, our sequence data represent strong evidence that the N- and C-halves of RM-PFK and the Bs-PFK subunit share a similar conformation. It follows that the tertiary structure of the RM-PFK subunit will be represented by two 'super' domains, each closely resembling the structure of the Bs-PFK monomer, joined by a connecting peptide of about 30 residues (Lys 320, N-half to Ala 5, C-half; Fig. 1). The tertiary structure of Bs-PFK and the location of catalytic and effector sites in the enzyme have been described by Evans and co-workers[9,10], and two schematic views of subunits in the Bs-PFK tetramer as related by the *x*- and *z*-dyad axes are shown in Fig. 2. Clearly, the N- and C-termini of both pairs of subunits are close enough to be joined covalently by the length of connecting peptide available. Therefore, the halves of the RM-PFK monomer could be disposed spatially as *z*- or *x*-dyad-related pairs of subunits in Bs-PFK (Fig. 2*a* and *b*, respectively). We

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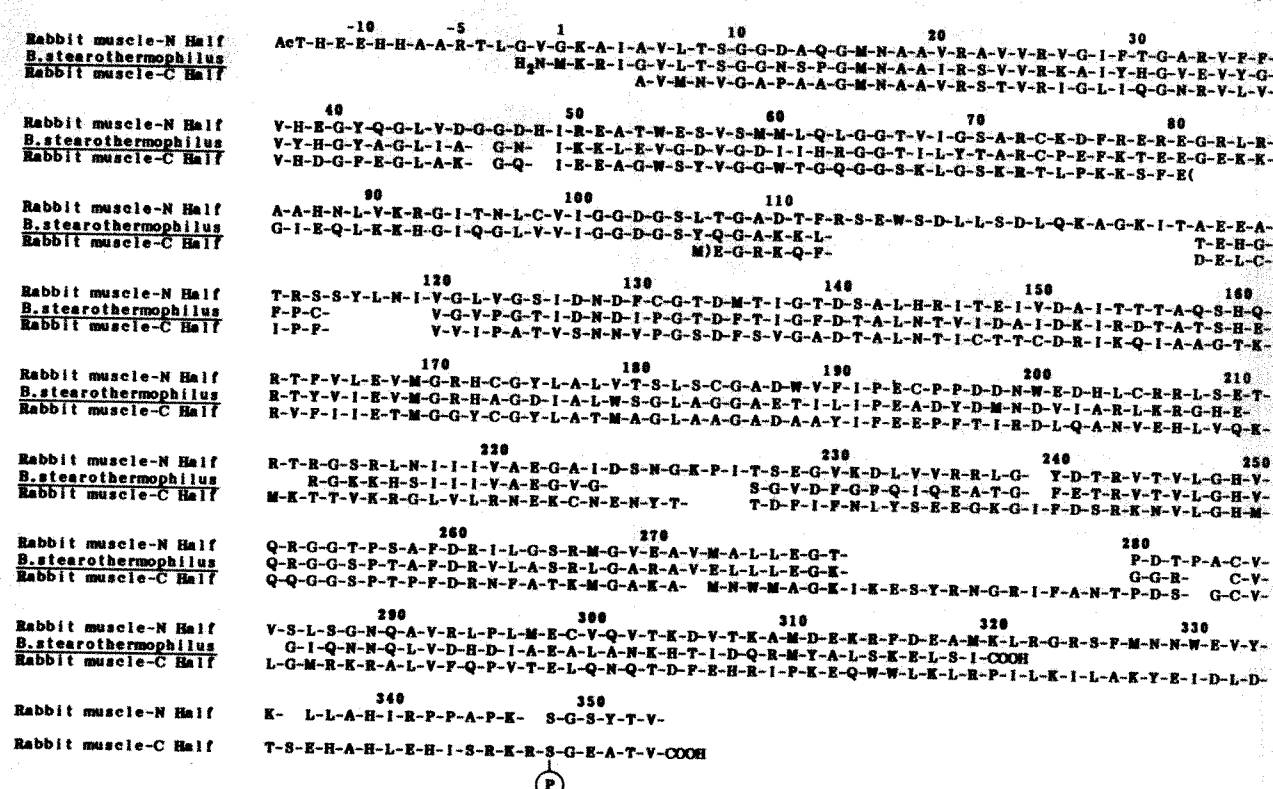
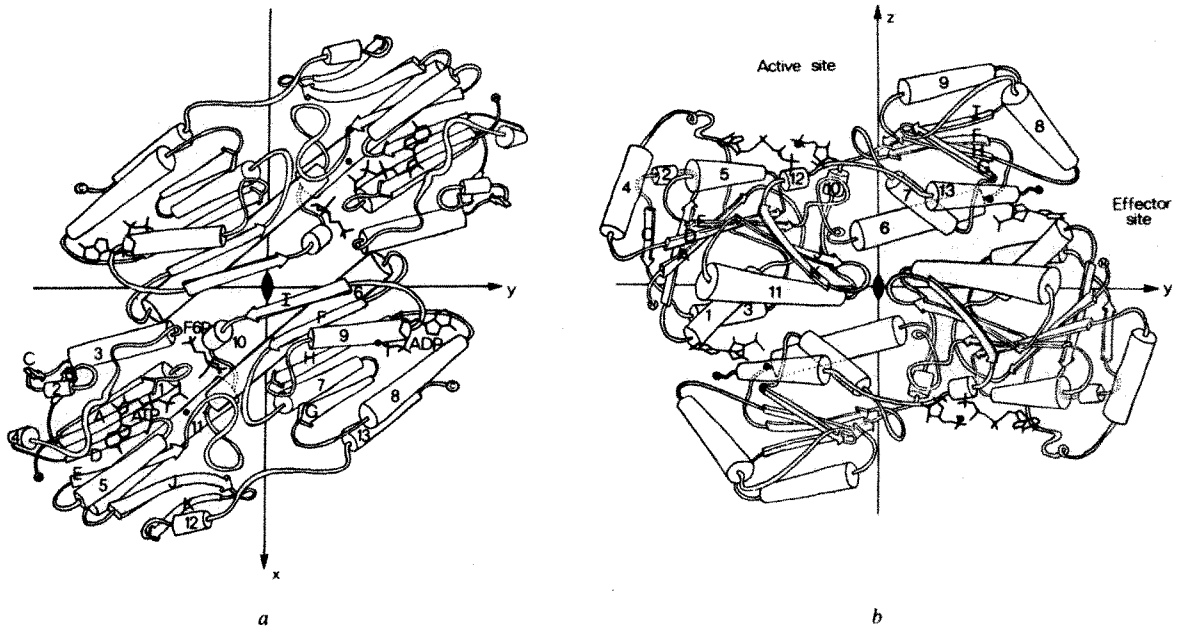


Fig. 1 The amino acid sequences of the N- and C-halves of RM-PFK compared with that of Bs-PFK. Numbering of residues is based on the Bs-PFK sequence[7] revised by crystallographic analysis[10] as follows: the tripeptide G–V–Y has been inserted after Y-35. In addition, V-43 and L-225 have each been changed to G; S-264 and A-265 have been reversed (P. R. Evans, unpublished). After residue 319, numbering is based on residues in the C-half. The Bs-PFK sequence is placed between the RM-PFK halves to emphasize homology. V-353 (N-half) is peptide-bonded to A-5 in the C-half of RM-PFK. S-348 (C-half) is the site of phosphorylation[20]. The region enclosed in parentheses (residues 81–106, C-half) is uncertain at present. Overlaps between N-half residues 50–51, 145–146, 162–163, 261–262 and 266–267 are based on homology and have yet to be proven.



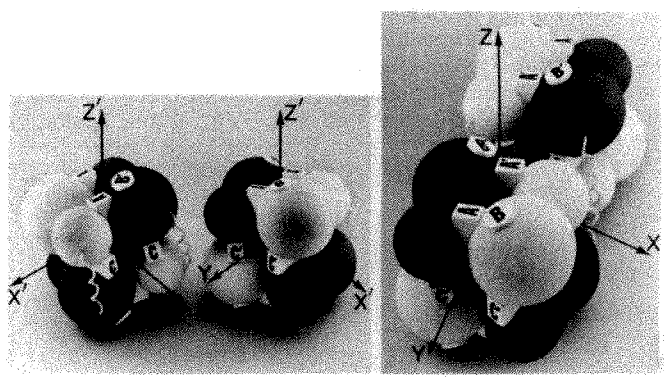
a *b*

Fig. 2 Schematic views of two subunits in the Bs-PFK tetramer: *a*, viewed along the *z*-axis; *b*, viewed along the *x*-axis. In each case, the other two subunits lie behind those shown. β-Strands are indicated by arrows (A–K) and α-helices by cylinders (1–13). The substrates ATP and F6P are shown in the active site and the activator ADP in the effector site. (Taken from Evans *et al.*[10].)

prefer the alignment depicted in Fig. 2*b*, giving an RM-PFK subunit in which the halves are related by the *x*-dyad. This preference is based on internal helical packing interactions which would stabilize the subunit, and the fact that cooperativity in binding of fructose 6-phosphate (F6P) occurs between *z*-dyad-related subunits in Bs-PFK[10]. Moreover, the dimer is thought to be inactive[13,14], as would be predicted by disruption of the F6P binding site. Fructose bisphosphate, like F6P, contributes to stabilization of the tetramer[14,15], and our view is that the FBP site is a mutated F6P site lying between the same *z*-dyad pair (see below). Covalent joining of the *z*-dyad subunits would create cooperative allosteric and active sites within, rather than between, subunits in RM-PFK, and we consider this mode of attachment to be less likely.

Speculation concerning the quaternary structural organization of RM-PFK is based on the structural relationship to Bs-PFK and the known D2 symmetry of the mammalian tetramer[16]. It is possible to bring two large RM-PFK subunits into juxtaposition to give a mammalian dimer equivalent to the Bs-PFK tetramer. Two views of such a dimer are represented by the models in Fig. 3*a*: here, the N- and C-halves of each subunit (light and dark, respectively) are joined by a connecting peptide (indicated by a helical strand of wire). The large and small lobes in each half represent the domains in Bs-PFK[9]; thus, the equivalent array of domains is preserved in our model of the RM-PFK dimer. In order to generate a tetramer having D2 symmetry, RM-PFK dimers must be brought together along the *y*-axis with all connecting peptides either on the outer or inner faces. Our preference for placing all the connecting peptides at the dimer interface (Fig. 3*b*) is supported by results from limit proteolysis. Treatment of native RM-PFK with subtilisin yields an inactive tetramer in which the N-terminal 50–60 residues have been removed[17], suggesting that the N-half of each subunit is pointing outwards and is susceptible to proteolysis, and that the intersubunit forces maintaining the tetramer are still intact. These forces may well involve interactions among the connecting peptides. We have also shown that of several enzymes tested, only trypsin and endoproteinase Lys-C cleave in the connecting peptide region. This argues in favour of our proposed shielded and symmetrical arrangement of these segments in the tetramer.

Crystallographic analysis of Bs-PFK has revealed the active site regions for binding the substrates F6P and ATP, and an allosteric site for the activator, ADP. These sites were designated



a *b*

Fig. 3 Models indicating our proposed structural organization of RM-PFK. Two views of the dimer and the tetramer are shown in *a* and *b*, respectively. The N- and C-halves of each subunit are light and dark, respectively, and connecting peptides are represented by helical strands of wire. Each side-by-side dimer is equivalent to the Bs-PFK tetramer[9], and the dimers are brought together to give a tetramer with D2 symmetry (*b*) in which all four connecting peptides are localized at the interface. The models also predict the location of sites for binding F6P (A), FBP (A'), ATP substrate (B), ADP activator (C), and ATP inhibitor (C'). Only one of the four C sites is visible in *b*; the other three are in corresponding positions at the interface. The remaining two C' sites are at the opposite end of the tetramer.

A, B, and C, respectively[9]. Sites which we believe have mutated from A and C are denoted A' and C' (see Fig. 3). Doubling of the Bs-PFK structure should give a RM-PFK tetramer with eight active sites and eight allosteric sites. Equilibrium binding studies have revealed, however, that there are only four catalytic sites in RM-PFK[18,19], and the greater number of effectors regulating the mammalian enzyme suggests that some catalytic and allosteric sites in the eukaryotic PFKs may have differentiated to provide new functionalities in these enzymes. Evidence in support of this notion derives from our comparative sequence analysis (Fig. 1). Homology in the vicinity of Asp-127 in Bs-PFK, a residue involved in F6P binding[10], is clearly evident in the N-half of RM-PFK but not in the C-half, where the corresponding residue is a serine. We believe, therefore, that the F6P site (A) is conserved as part of the catalytic apparatus of RM-PFK,

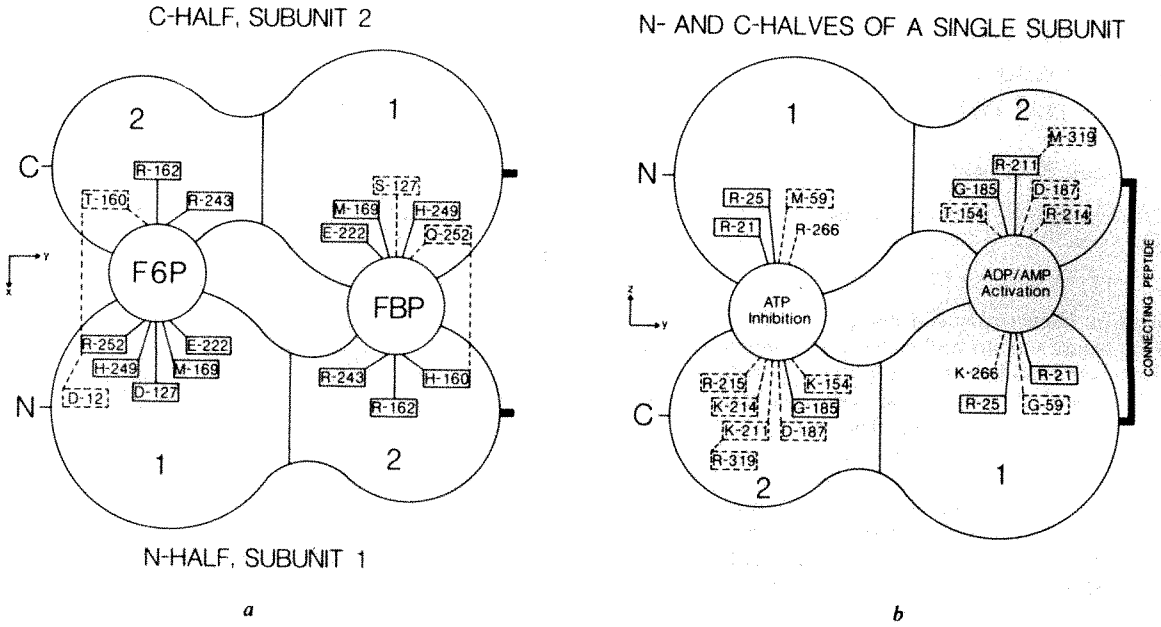


Fig. 4 Location of substrate and effector sites in RM-PFK based on crystallographic analysis of Bs-PFK and comparative sequence analysis. Residues enclosed in solid boxes are identical to those at corresponding positions in Bs-PFK; those enclosed in dotted boxes are different. *a*, View along the *z*-dyad axis of the N- and C-halves of two adjacent subunits which together define sites for cooperative binding of F6P (site A) and FBP (site A'). Dotted lines between residues indicate possible hydrogen bonds[10]. *b*, View along the *x*-dyad axis of the halves of a single RM-PFK subunit in which residues presumed to be involved in binding ADP activator (C site) and ATP inhibitor (C' site) are noted (compare with Fig. 3). The solid bar labelled 'connecting peptide' represents the covalent bridge between the halves related by the *x*-dyad axis (Figs 2*b*, 3). Position 266 is not boxed because its possible involvement in ligand binding was suggested by chemical modification[21] and not by crystallography.

but that its counterpart in the C-half (A') now binds FBP (Fig. 4*a*). This seems reasonable as Asp 127 is probably required as a base catalyst acting to increase the nucleophilicity of the O-1 hydroxyl of F6P for attack on the γ-phosphate of ATP. Its presence would cause electrostatic repulsion of FBP; replacement of Asp 127 by Ser in the new A' site would remove this negative charge and provide more room for binding of the fructose 1,6- and 2,6-bisphosphates which are known to compete for a common site[15]. Thus, it is reasonable to suggest that the structural change leading to replacement of the catalytically essential Asp 127 has led to loss of a catalytic site (A) and creation of an allosteric site for FBP activation (A').

The arguments thus far account for the presence of four catalytic sites in the RM-PFK tetramer, and provide evidence as to how four new sites for binding FBP may have arisen in evolution. Furthermore, the cooperativity in binding of F6P and FBP is in keeping with our model of the tetramer where binding is between subunits (Figs 3*b*, 4*a*). The case is not so clear-cut for a possible mutation of the ADP activation site (C) to a new site for ATP inhibition (C'). Here the inferences rest more on results from limit digestion with subtilisin[17] than on comparative sequence analysis. Although the subtilisin-treated RM-PFK tetramer retains the ability to bind FBP and ADP, it no longer binds ATP at the inhibition site, nor F6P. These results for a tetrameric PFK derivative having degraded N-termini are consistent with our location of F6P and FBP sites, A and A', respectively (Figs 3*b*, 4*a*). They also lend credence to the idea that the ADP activation site (C) involves residues in each subunit that are protected from the proteinase, that is, in the middle or C-terminal regions far enough removed from the N-terminal segment (Fig. 4*b*). Accordingly, the ATP inhibition site (C' in Fig. 3) could be a mutated ADP activation site, composed of N-terminal amino acid side chains, that is destroyed by subtilisin (Fig. 4*b*). We suggest, therefore, that RM-PFK has retained four of the ADP activation sites of its bacterial progenitor, but that it has gained, through evolution of the remaining four possible ADP sites, four new ATP inhibition sites per tetramer.

Thus, our model of RM-PFK predicts that the N- and C-halves of each subunit in the tetramer are equivalent in tertiary and quaternary structural organization to the subunits in a hypothetical Bs-PFK octamer composed of two bacterial enzyme tetramers joined end-to-end along the *y*-axis. The A sites for F6P binding and the C' ATP inhibition sites are exposed and comprise side chains from residues in the N-terminal regions, while the A' and C sites which bind FBP and ADP activators, respectively, are made up of residues that are internal, or C-terminal in the subunits which are protected from digestion with subtilisin. At present we have no crystallographic data pertaining to the subunit organization or functional sites of RM-PFK. However, our results obtained by comparative sequence analysis, together with findings from X-ray crystallography of Bs-PFK, chemical modification studies, kinetic analysis and limit proteolysis, can be interpreted to give a unified tentative working model of the mammalian enzyme tetramer which can be addressed experimentally.

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Successive incorporation of force-generating units in the bacterial rotary motor

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Mot **mutants of *Escherichia coli* are paralysed: their flagella appear to be intact but do not rotate[1]. The *motA* and *motB* gene products are found in the cytoplasmic membrane[2]; they do not co-purify with flagellar basal bodies isolated in neutral detergents[1]. Silverman *et al.* found that *mot* mutants could be 'resurrected' through protein synthesis directed by λ transducing phages carrying the wild-type genes[2]. Here, we have studied this activation at the level of a single flagellar motor. Cells of a *motB* strain carrying plasmids in which transcription of the wild-type *motB* gene was controlled by the *lac* promoter were tethered to a glass surface by a single flagellum. These cells began to spin within several minutes after the addition of a *lac* inducer, and their rotational speed changed in a series of equally spaced steps. As many as 7 steps were seen in individual cells and, from the final speeds attained, as many as 16 steps could be inferred. These experiments show that each flagellar motor contains several independent force-generating units comprised, at least in part, of motB protein.**

Cells of a *motB* inducible strain were tethered to a coverslip, exposed to a *lac* inducer, and viewed with a microscope linked to a video recorder. The rotational behaviour of one such cell is analysed in Fig. 1. The cell remained in roughly one position for 16.3 min following the addition of inducer, executing brownian motion with a fixed mean orientation about its point of attachment. Then it suddenly began to spin, gaining speed over a period of ~17 min before levelling off at a constant speed. Abrupt changes in rotation rate were evident, particularly at the slowest speeds. Most cells in the population of 32 studied started to spin between 5 and 8 min from the time of induction; these latencies ranged from as little as 3 min to over 25 min. The time spent at the lowest speed was highly variable, ranging from a few seconds to a few minutes. The cell of Fig. 1 started spinning counterclockwise (as viewed with the cell body in front of the tethering surface, the direction corresponding to smooth swimming[4]), and, as the rotation rate increased, spun alternately clockwise (CW) and counterclockwise (CCW). The directional bias (the probability of spinning CCW) of the cell was unity at the lowest speed, but it fell to a lower value soon afterwards and remained relatively constant for the remainder of the experiment. The reversal rate was zero at the lowest speed, then increased dramatically during the next few minutes and levelled off long before the cell reached its maximum speed. The asymptotic values for directional bias and reversal rate were similar to those found for wild-type cells[5,6]. Cells rarely changed direction at the slowest speed: only two of the cells studied showed brief CW intervals at this level, but all reversed frequently at higher levels. At any given level, cells that reversed spun CW or CCW at the same speed.

Figures 2, 3 document stepwise changes in rotation rate for two more cells. A rapid doubling of rotation rate was characteristic of the first transition: for 15 cells in which this transition was clearly resolved, the mean speed ratio (±s.e.) was 2.05 ± 0.08. Note that the cell of Fig. 3 underwent transitions that either increased or decreased the speed by one level, spinning at four different levels over the interval shown. Evidently, the incorporation of functional force-generating units is not irreversible.

By arbitrarily assigning the initial rotation rate of a cell to level 1, the next higher rate to level 2, etc., a set of levels was derived for each cell. Figure 4 plots mean rotation rate against level number for four representative cells in which multiple levels were observed. As many as seven distinct levels (excluding

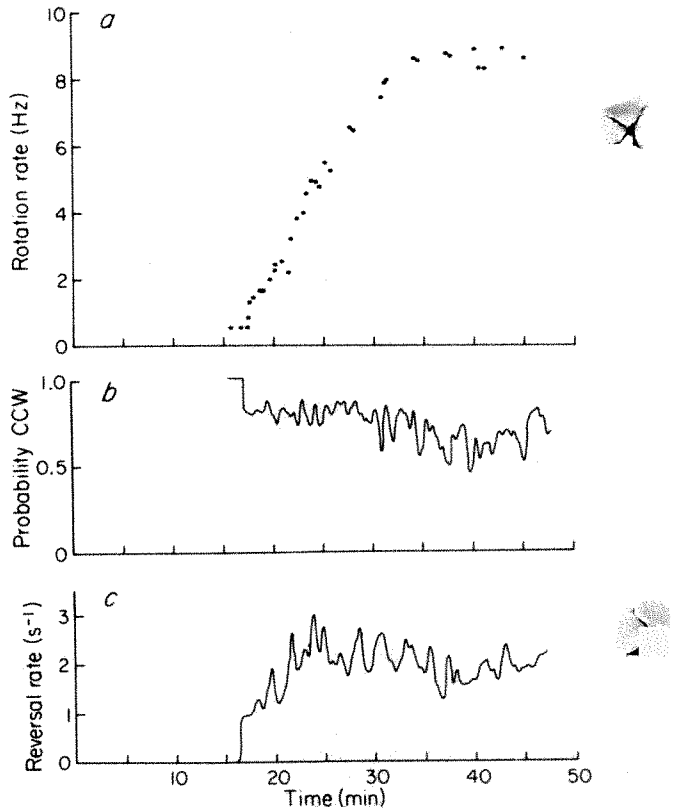


Fig. 1 Rotational behaviour of a single tethered cell, following initiation of transcription of the wild-type *motB* gene at time $t = 0$. *a*, Time course for induction of rotation; *b*, directional bias; *c*, reversal rate. The points in *a* show the average rotation rate computed from 30–300 revolutions registered by computer. Directional bias and reversal rate were determined as described previously[5,6]. The cell began to spin at $t = 16.3$ min at 0.53 ± 0.03 Hz (mean ± s.e.m. for 33 revolutions). It levelled off at $\sim t = 33$ min at 8.57 ± 0.07 Hz (mean ± s.e.m. for last nine data points).

Methods: Cells of strain HB154 (see below) were grown in minimal medium, washed and tethered in motility medium, as described previously[6], except that ampicillin (50 μg ml^{-1}) and tetracycline (10 μg ml^{-1}) were present in all media. At $t = 0$, the motility medium was replaced by a minimal medium containing glycerol and essential amino acids[19], as well as 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG)[20]. In subsequent experiments, the cells were exposed to this medium (without IPTG) 10 min before the addition of the inducer; this appeared to increase the number of cells that responded without changing the kinetics of their induction. The flow cell was mounted on the temperature-controlled stage (32 °C) of an inverse phase-contrast microscope (Nikon Optiphot with a BM ×40 objective) linked to a video camera (Ikegami ITC-47; 2:1 interlace) and recorder (Panasonic NV-8950, VHS format). A digital time display (accurate to 1/60 s, a single video frame) was superposed on the video image[21]. The speed analysis of the videotaped records is described in Fig. 2 legend. Construction of *motB*-inducible strain HB154 began with plasmid pGM1, a pBR322 derivative carrying ampicillin resistance and a restriction fragment of the *mocha* operon[22] fused to *lac*Zp, originally produced to direct *motB* overproduction[18]. The fragment contained the C-terminus of the *motA* gene and a complete copy of the *motB* gene. A λgt1–pGM1 hybrid phage was made by cutting the plasmid and the phage at their unique *Eco*RI sites, ligating the mixture, and using the resultant constructs to infect *E. coli* strain RP3087 (an AW405 derivative[23] prepared by J. S. Parkinson carrying the *motB*^{580} allele[1] and deleted for λ-att) in the presence of ampicillin). An ampicillin-resistant transductant was selected which was lysogenic for λ; this cell presumably maintains the phage in a plasmid state using the pBR322 origin of replication. An F′ element carrying *lacI*^{Q} was then crossed into this strain to repress the *lac* promoter[24]. The hybrid phage copy number was reduced by adding a second plasmid, pMB9, bearing tetracycline resistance[25], and the transformed strain, designated HB154, was maintained under double antibiotic selection. This strain was immotile in the absence of a *lac* inducer; when tethered, less than 3% of cells showed any evidence of rotation.

level 0) were resolved using the video technique. For objects the size of bacteria, the angular rotation rate is strictly proportional to the applied torque; therefore, the linearity of these plots implies that the steps in torque associated with transitions between levels are equal. Evidently, each mot protein, or protein complex, contributes the same increment of torque to the flagellar motor, at least for the incorporation of up to seven force-generating units. Unfortunately, most cells in which multiple levels could be measured came off the glass or became stuck to it, prematurely terminating the experiment at speeds ranging from ~2 to 8 Hz.

The cell of Fig. 1 was an exception; the rotation rate of the first level was measured accurately, and the cell continued to spin until its speed reached a maximum value. In this case, the ratio of the final to the initial speed (16.2 ± 0.8) provides an estimate of the total number of force-generating units in the intact motor. If the efficiency of the flagellar motor remained constant over the range of speeds covered (as argued below) and all the force-generating units continued to operate independently, the motor in this cell contained about 16 such units. We also compared the mean rotation rate at level 1 for the population of cells studied here (0.76 ± 0.08 Hz; mean ± s.e.m. for 14 cells) with that of fully energized wild-type cells used in a previous study[7] (11.20 ± 0.46 Hz; mean ± s.e.m. for 33 cells). The ratio of these means (±s.e.) is 14.7 ± 1.7, again suggesting that each flagellar motor could contain up to 16 independent force-generating units. Sixteenfold rotational symmetry has been found for an M-ring of the *E. coli* flagellar motor[8]. This is also the number (usually 15, sometimes 14 or 16) of membrane particles ('studs') seen at the periphery of annular depressions in freeze-fracture electron micrographs of the cytoplasmic membrane at the flagellated poles of another bacterium, *Aquaspirillum serpens*[9].

The data of Fig. 4 do not indicate the existence of a rotational threshold: each curve extrapolates to the origin. It is possible

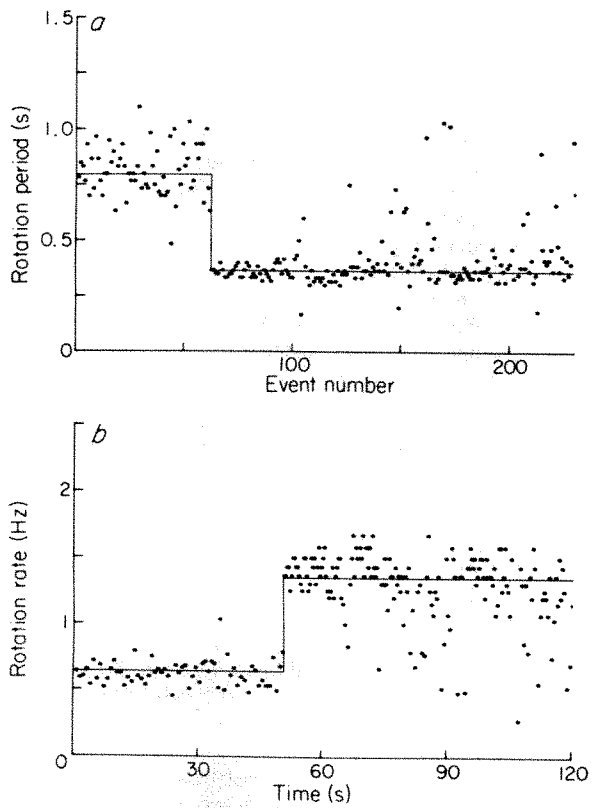


Fig. 2 Stepwise doubling in rotation rate of a tethered cell induced for motB synthesis. *a*, The first 280 rotation periods, plotted sequentially; *b*, rotation rate as a function of time computed from these periods. The solid lines represent the mean periods or rates for different intervals; see below. The cell started to spin 6.9 min after addition of inducer and doubled its rotation rate 52 s later, jumping from 1.28 ± 0.08 Hz (mean ± s.e.m. for 61 revolutions) to 2.67 ± 0.10 Hz (mean ± s.e.m. for 166 revolutions), a factor of 2.09 ± 0.15. The transition was completed in less than four video frames (67 ms), our limit of resolution.

Methods: The rotation periods were measured with a device linked to a computer (Apple II$^+$, containing a California Computer Systems 7440A Programmable Timer card connected to custom-built electronics) that generated a pulse whenever the image of a particular cell crossed a video cursor. The intervals between successive passages were stored in arrays for subsequent analysis. All of these data points are shown in the figure. The instantaneous rotation rate was computed from the reciprocal of each rotation period. Note that the points at large rates tend to fall on 'layer lines'—this reflects the fact that video images are recorded at 60 frames s^{-1}; the time intervals are constrained to be multiples of 16.7 ms. This limitation is significant when the rates are high. As the cells often changed direction, some intervals did not correspond to complete revolutions of the cell body. These intervals were shorter than adjacent intervals if the image changed direction within half a cycle after crossing the cursor; otherwise they were longer. Also, cells had a tendency to stick to the glass; when this happened, the measured period was abnormally long. An interactive graphics program allowed the operator to identify and eliminate most of these events from calculations of mean periods or rates. The standard error for each mean was obtained from the root mean square deviation from the mean of the edited distribution; this deviation was divided by the square root of the number of rotation periods and then adjusted upwards to compensate for the truncation due to editing (assuming that the parent distribution was gaussian).

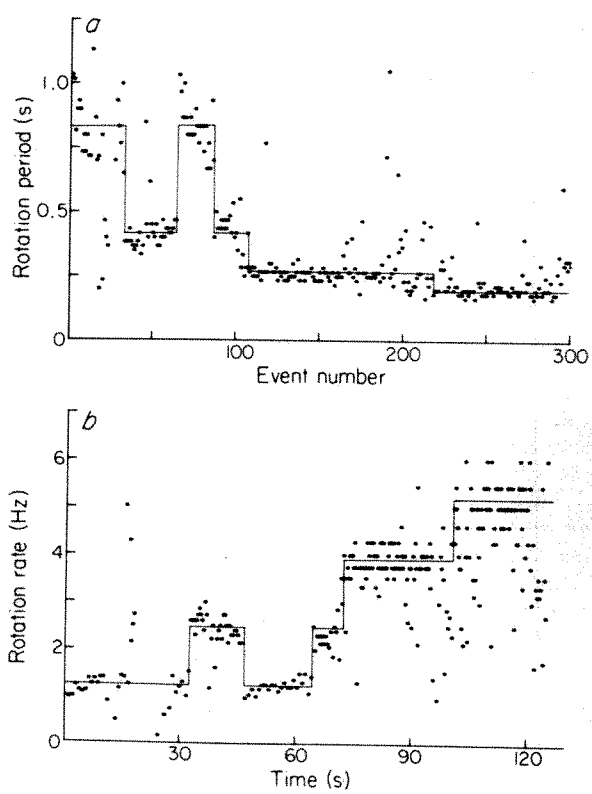


Fig. 3 Stepwise changes in rotation rate of another tethered cell induced for motB synthesis. *a*, The first 300 rotation periods, plotted sequentially; *b*, rotation rate as a function of time computed from these periods. The solid lines represent the mean periods or rates for different intervals, computed as described in Fig. 2 legend. The cell started to spin 5.9 min after the addition of inducer. Mean rotation periods tended to decrease in a harmonic series (as $1/n$, with n an integer); the corresponding rotation rates increased in an arithmetic series (as n). For large n, transitions between rotational periods of different mean amplitude became increasingly difficult to discern; as a result, the scatter in rotation rates became relatively more severe.

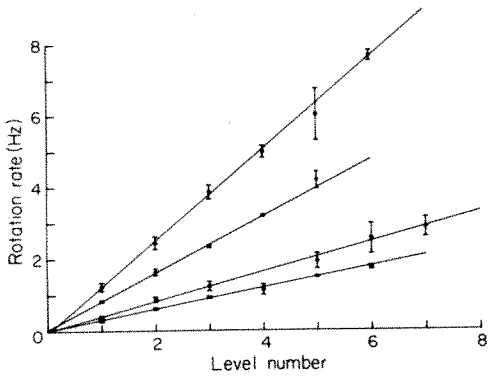


Fig. 4 Dependence of rotation rate on level number for four different cells. The vertical bars show the s.e.m., computed as described in Fig. 2 legend, and the solid lines are weighted least-squares linear fits, not constrained to pass through the origin. The different slopes reflect the variations in rotational drag from cell to cell (see text).

that the first transition that we have observed is not between levels 0 and 1, but rather between higher levels, say, 1 and 2. The possibility that a fully energized cell has no threshold is ruled out by the observation that the mean position of a paralysed cell (or flagellar filament[10]) remains fixed before the onset of rotation. It could be that the barrier to rotation in a fully energized cell is abolished by the introduction of functional mot protein. This question can be resolved by measuring speed as a function of protonmotive force in cells having different fixed numbers of force-generating units.

Given the increment in rotation rate from level to level, it is possible to infer the torque generated by a single force-generating unit. The constant of proportionality relating the torque generated by the motor to the rotation rate of the cell is the viscous drag coefficient, which depends only on the geometry of the cell, its position relative to the tethering surface, and the viscosity of the external medium[11]. Drag coefficients were estimated by assuming that cells behave as cylinders with hemispherical endcaps, rotating about an axis through the centre perpendicular to the long axis[12]. We neglected the interaction with the tethering surface, which is small[13], and the fact that cells are often tethered towards one end. The latter effect can increase the viscous drag by more than a factor of two[13], thus the drags calculated here represent an underestimate. The increments in torque from level to level for different cells appeared to be distributed normally with a mean (±s.d.) of $2.7 \pm 0.8 \times 10^{-12}$ dyn cm (11 cells). This value may be considered an approximate lower bound for the torque associated with a single force-generating unit; the torque is approximately the same for all units in all cells.

Given this result, it is possible to calculate the number of protons passing through a force-generating unit in one revolution. The energy balance for the motor can be written as $2\pi M = \varepsilon N e \Delta p$, where M is the torque, e is the electron charge, Δp the protonmotive force, N the total number of protons per revolution passing through the motor, and ε the motor efficiency, which could vary, for example, with angular velocity or protonmotive force. The left-hand term is the work done per revolution in driving the cell around. The right-hand term is the total work available from the proton gradient times the efficiency. Experimental evidence that the flagellar motor runs at constant torque at low speeds[14,15] implies, for these motors, that the product εN is constant. For any tightly coupled motor model, in which the passage of protons through the cell membrane is obligatorily coupled to rotary motion, N is constant. Given the experimental evidence for constant torque, this implies that ε is constant. Furthermore, if the motor in such a model is non-dissipative, that is, if it functions reversibly with no energy going into heat, then the efficiency will be unity.

A model of this kind has been proposed in which force is generated by the interaction of n proton channel complexes (independent force generators) situated around the periphery of a disk bearing m proton-binding sites[16]. The number of protons passing through a single channel complex in one revolution is m. Assuming unit efficiency, the torque generated by this channel complex can be written as $M = me\Delta p/2\pi$, where it is assumed that the efficiency is one. Given this torque and the protonmotive force, with $M \geq 2.7 \times 10^{-12}$ dyn cm and $\Delta p = 162$ mV (ref. 17), we estimate m to be ≈65. The total number of protons passing through a motor with n channel complexes in one revolution is $N = nm$. Assuming that the full complement of force generators is 16, N is of the order $16 \times 65 = 1{,}040$, in close agreement with an earlier estimate based on similar assumptions of tight coupling and zero dissipation[11].

The *motB* gene product is a peptide of molecular weight ~39,000 which is known to assemble in the cytoplasmic membrane without proteolytic cleavage; this mode of insertion has been confirmed in minicell preparations which are unable to synthesize any other flagellar proteins[18]. Here we have found that the rotation rate of tethered, paralysed cells increased in a stepwise fashion concomitant with motB synthesis. The simplest explanation for this result is that the wild-type motB peptides diffuse to the vicinity of the flagellar motor and act there, either alone or in conjunction with other components (for example, motA), as independent force-generating units. We cannot exclude the possibility, however, that the motB protein activates some other component that, in turn, incorporates as a force-generating unit. Since the rotation rate occasionally decreased in stepwise fashion, incorporation is reversible. The chromosome of the strain that we used carried a point mutation in *motB*; therefore, defective copies of motB protein might have been present before induction. In that event, the addition of functional force-generating units could have involved displacement rather than simple incorporation. As many as 16 force-generating units can be accommodated—it is attractive to suppose that these are transmembrane particles comprised of motA and motB protein[15,16].

We thank Chris Kaiser for the construction of strain HB154, which made this work possible; other strains were provided by Gail Mandel and Sandy Parkinson. We thank Felice Borisy for technical assistance; Markus Meister and Mel Simon for helpful discussions; and Shahid Khan, Jeff Segall, Pat Conley and Bob Smyth for their comments on the manuscript. This work was supported by US NSF grant PCM-8215126. S.M.B. acknowledges support of a Proctor and Gamble Fellowship and from the National Research Service Award Program of the NIH.

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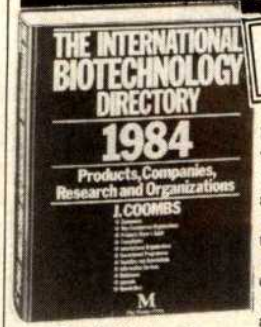
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by **E.S. Albone,** *The Science School, Clifton College, Bristol*

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Inflationary cosmology

Gary Steigman

The Very Early Universe.
Edited by G.W. Gibbons, S.W. Hawking and S.T.C. Siklos.
Cambridge University Press: 1983. Pp.480. £25, $49.50.

THE "standard" hot big bang model provides a good framework for understanding the early evolution and present structure of the Universe. The Robertson–Walker–Friedman (RWF) models are isotropic, homogeneous solutions to Einstein's gravitational equations in the presence of matter (nucleons) and radiation (microwave photons). Penzias and Wilson's discovery of the cosmic background radiation provided dramatic confirmation that these simple models could describe the early history of the Universe, and ushered in the "modern" era in the study of cosmology. The blackbody spectrum of the microwave background is established by electromagnetic interactions during the early evolution of the Universe and preserved during its later evolution by the homogeneity and isotropy of the RWF models. Furthermore, nuclear and weak interactions occurring during the first few minutes in the evolution of the Universe lead to the synthesis of the light elements, the abundances of which are observed to be in excellent agreement with the predictions of primordial nucleosynthesis in the standard (RWF) model. As for the more recent evolution, small deviations from the perfect homogeneity of the RWF models grow with time and may account for the observed large-scale structure of the Universe (galaxies, clusters, voids and so on).

The simplest hot big bang model has proved to be remarkably efficacious; to ignore its successes would be churlish. Nonetheless there are problems. Many questions and puzzles are neither answered nor addressed within the context of the standard model. For example, the "Planck time" $t_p \approx (G\hbar/c^3)^{1/2} \approx 10^{-43}$s is a "natural" timescale for cosmology. The present Universe is very old ($t_0 \approx 10^{10}$ yr $\approx 10^{60} t_p$) and yet very young in the sense that the spatial curvature is not yet dominant; our Universe is very "flat". The response of the standard model to the age–flatness puzzle is to appeal to initial conditions: the Universe is the way it is because it was the way it was.

A different problem is posed by the matter content of the Universe. The observed Universe consists overwhelmingly of matter; antimatter is absent. Why? Early on there must have been a tiny excess of matter over antimatter so, when nucleon–antinucleon annihilation ceased, a trace amount of relic nucleons survived. The present ratio of nucleons to photons (η) is a measure of the requisite excess: $10^{-10} \lesssim \eta \lesssim 10^{-9}$. Within the standard model there is no explanation for the magnitude of η (or even why it should not be zero); η is the size it is because it was the size it was.

Even the observed large-scale homogeneity, a key ingredient of the standard model, is a serious puzzle. The finite speed of light guarantees the existence of particle horizons so that the various parts of the Universe from which the microwave radiation originates were never in causal contact. Why, then, is the flux observed to be so very uniform? Why, indeed, is the large-scale Universe so very homogeneous?

There are still further questions. Why is the cosmological constant so small? Is it zero? What of the singularity of the RWF models? The list goes on, but all is not darkness and despair. Less than a decade ago the tide of a revolution in cosmology began to swell. Today it has risen to a flood. Its source was high-energy physics. The successful unification of the electromagnetic and weak interactions led to the predictions of new phenomena such as neutral current interactions; the gauge theory of the strong interaction predicted especially simple behaviour at high energy (asymptotic freedom); Grand- and Super-Unified theories were proposed predicting new phenomena and new particles, often at energies far beyond or rates far below those attainable in the laboratory. Some cosmologists and a few particle physicists quickly appreciated that the early Universe was the ultimate accelerator — the particle physics laboratory of last resort. That particle physics might provide the key to solving various cosmological puzzles in the early evolution of the Universe was much less obvious. Yet the relationship between particle physics and cosmology which has blossomed in recent years, while not entirely trouble free, has been a remarkably fecund affair.

For example, a solution to the asymmetry puzzle is provided by Grand Unified Theories (GUTs) in the context of the very early evolution of the Universe. In most GUTs, baryon number is not conserved and charge (C) and charge-parity (CP) conservation are violated. As Sakharov noted in his recipe for generating a baryon asymmetry, these are two of the three required ingredients. The third, deviation from equilibrium, is provided by the expansion of the Universe. Although there are too many free parameters in the GUTs to permit a calculation of the nucleon to photon ratio, η, from first principles, it is nonetheless clear that a framework for this calculation now exists. No longer need we appeal to initial conditions: η is what it is because of baryon number and C and CP violating interactions which occurred during the very early evolution of the Universe. The universal baryon asymmetry and the instability of the proton are two sides of the same coin.

Lest we be lulled into a false sense of security, it must be said that the same GUTs which solve the asymmetry puzzle, predict the existence of superheavy ($\sim 10^{16}$ GeV) magnetic monopoles. In the standard model these monopoles are produced during the same epochs in which the baryon asymmetry is generated and they survive in abundances comparable to that of the nucleons. The enormous mass density in the relic monopoles would have driven the Universe to expand so rapidly that today, when the blackbody radiation is at a temperature of 3K, the Universe would be only $\sim 10^3$ yr old! (It is often stated, incorrectly, that relic monopoles "close" the Universe. The curvature neither knows nor cares about the stuff of the Universe.) So, GUTs and cosmology solve the baryon asymmetry puzzle but present us with a new problem — relic monopoles; failure snatched from the jaws of success!

With the monopole problem as one of its catalysts, there then occurred a revolution within the revolution. The cosmological constant emerged from the closet to play a significant role during the very early evolution of the Universe. As Zeldovich and others have noted, the cosmological constant (Λ) may be regarded as the energy density of the vacuum ($\Lambda \equiv 8\pi G\varrho_v$). Although Λ is small today ($\Lambda < H_0^2$, $\varrho_v < 10^{-4}$eV cm^{-3}), the energy density of the vacuum would have been enormous during the very early evolution of the Universe ($\varrho_v \sim 10^{110}$–10^{126} eV cm^{-3}). K. Sato and A. Guth realized that the vacuum energy density would drive the early evolution of the Universe and Guth appreciated that the "inflationary" exponential expansion would, in one fell swoop, solve a myriad of cosmological conundrums. Cosmology hasn't been the same since.

In the summer of 1982, Stephen Hawking, with the support of the Nuffield Foundation, organized what turned out to be a very productive workshop in Cambridge, UK, on "The Very Early Universe". Truly international in character — the participants came from Great Britain, continental Europe, the United States and the Soviet Union — the meeting covered topics as diverse as quantum gravity and galaxy formation, monopoles, strings and massive neutrinos, photinos and axions, supersymmetry, GUTs and the cosmological constant. Not surprisingly, the "new" inflationary models of Linde and of Albrecht and Steinhardt were the centre of interest. And rightly so, for, if it could be implemented, new inflation would solve the age, flatness,

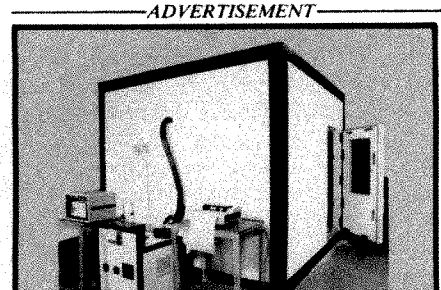
homogeneity and monopole problems. Furthermore, the reheating after the inflationary epoch would then account for the enormous entropy ($\gtrsim 10^{87}$ photons in the observable Universe) as well as the large entropy per baryon ($\eta^{-1} \gtrsim 10^{9}$ photons per nucleon) of the Universe. What is more, new inflation predicts perturbations of the spectral form first suggested by Harrison (and, therefore, now known as the Zeldovich spectrum!). The workshop was particularly noteworthy in that the participants arrived emboldened by recent successes and left with further successes and new problems with which to wrestle. The struggle still continues.

This book, a record of the workshop, provides a window on the excitement of the meeting itself. Virtually all the articles are interesting, comprehensive and well written. Several are, and will remain, valuable reviews of an entire subfield of cosmology or particle physics. Although some are highly technical, most can be tackled by the knowledgeable graduate student; cosmologists will find the particle physics articles comprehensible and vice versa. Especially worth reading are the introductory and summary reviews by Wilczek; the cosmology article (virtually a monograph on modern cosmology) by Rees; the primer on GUTs and Supersymmetry by Ellis; the self-contained introduction to and review of monopoles by Preskill; the inflationary articles by Guth, Linde, Steinhardt and Hawking; and the outlines of the development of perturbations in the inflationary models by Turner, and by Lukash and Novikov. I have learned a great deal from these articles and expect to return to them frequently in my teaching and research. Conspicuous by its absence, however, is a contribution by Starobinsky who, to judge from the comments of many of the participants, played a major role in discussions at the meeting.

Stephen Hawking is owed a debt of gratitude for having attracted the right people to the right workshop at the right time. In *The Very Early Universe* Hawking and his co-editors, G.W. Gibbons and S.T.C. Siklos, have provided the astro–particle physics community with a valuable work of reference. □

Gary Steigman is Professor of Physics in the Bartol Research Foundation of the Franklin Institute, Newark, Delaware, and Adjunct Professor in the Physics Departments at the University of Delaware and the University of Pennsylvania.

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Accounts of energy

Colin Sweet

The Economics of Nuclear Energy.
Edited by L.G. Brookes and H. Motamen.
Chapman & Hall/Methuen: 1984.
Pp.420. £27.50, $65.

ATTITUDES towards nuclear power in one country have always been more influenced by developments elsewhere than is the case with any other industry, with the possible exception of the defence industries. It is appropriate, therefore, that this new addition to the literature should set out to be international in scope. The 23 authors are drawn from six countries.

Despite its title, the book is not in any sense an economics text. Nor is it a straightforward exercise in applied economics. Rather, it is series of essays on different aspects of nuclear power as seen from different countries. This approach certainly has its merits, but to contribute something more to the current debate the book would have had to have been explicitly eclectic. As it is, while the authors have different ways of making their economic assessment (such that one cannot often be compared with another), the conclusion that they all arrive at, with one possibly neutral exception, is that nuclear power is the most attractive option on offer for future growth in electricity generation.

The first three chapters of the book are concerned with a systems analysis of electricity supply and are in many ways the most satisfactory. They provide a good basis for discussion of economic pricing. One point which emerges very clearly is the great complexity of the nuclear–electric system, from the mining of uranium to the delivery of the product to the consumer. And between them, these chapters — by C. Starr and O. Yu (United States), L. Gouni (France) and T. Berrie (UK) — bring out well the technical constraints of electricity systems generally. Especially for the layman this is good start. However the treatment is not without problems which, once raised, recur in the so-called economic discussions of the subject. Starr and Yu conclude that centralization of production is a logistical objective. Gouni follows by drawing attention to the monopolistic tendency that accompanies such centralization. But like his counterpart in the UK, J. Rhys (Electricity Council), Gouni does not really face the contradiction between this reality and the assumptions of a competitive market. The result is that Rhys's chapter in particular, which is one of the few that tackles the problem of economic pricing, has an overly academic air. His conclusion that "In the long run, provided nuclear power can be established as the cheapest means, it is likely to be the dominant factor in determining the overall level of electricity prices" may be conceptually a narrow basis for making a judgement but it is about the most open-ended statement to be found in the book.

One reason why the book is methodologically weak is that it rests too much on the thinking that prevailed when nuclear economics was inseparable from propaganda of the institutionalized kind. It is not the authors' fault that their book went to press before they had time to absorb the massive amount of information put before the Sizewell "B" Public Inquiry. Nevertheless it is unfortunate. In their submissions, the Central Electricity Generating Board appear finally to have put historic cost accounting behind them for purposes of investment appraisal and to have embraced opportunity cost analytical studies. This is something which British authors have to communicate to their opposite numbers in nuclear establishments in other parts of the world. It is a pity that here the opportunity was missed.

Interestingly enough, the best comparative study of nuclear and coal-fired generation comes from D. Schmitt and H. Jung of Cologne University. They use the requirement revenue method which computes likely discounted costs of future projects against future revenues. The cost assumptions are not always convincing and the treatment of the demand side is conspicious by its absence (on this point they could do well to read what Gouni has to say about the demand factor). They are however aware of the limitations of such economic exercises and indeed they make the observation which one would like to have seen feature more prominently in other contributions — that the future of nuclear power will not be decided by economics. "The crucial barrier is public

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acceptance" as far as Schmitt and Jung are concerned, and that means that "The expansion of nuclear energy thus becomes a problem of political decision making". They are surely right.

The least satisfactory chapters in the book are one from the United States and two others from France. The American study, by G.R. Corey, is the familiar full cost analysis which abounds in the past literature. It is replete with data that can hardly be checked and assumptions that are heroic at nearly all points. The contributions from the French Atomic Energy Authority (CEA) are the worst kind of institutional arrogance. This is a great pity, but not a surprise: the French commitment to nuclear power is not only greater than that of any other country, but their programme is beset with considerable problems of overcapacity and cost. J. Baumier and L. Duchatelle, and their colleague L. Thiriet (appropriately from the public relations department), have no wish to explore problems. Baumier and Duchatelle write about the French Fast Breeder Reactor, Super-Phénix, which from an engineering point of view may prove to be an achievement. But from the economic standpoint it is a disaster. The attempt by the authors to juggle with figures in order to show that while the first station will produce power at twice the cost of a Pressurized Water Reactor the "cost ratios will progressively approach unity" is unconvincing and ought not be taken seriously (as I am sure it is not by Electricité de France).

Less strident but characterized by the same cover-up technique is the contribution by A. Farmer of the United Kingdom Atomic Energy Authority. The recent stinging report by the Comptroller and Auditor General on the vast sum of money that has been expended on the UK Prototype Fast Reactor at Dounreay, which nine years after completion hardly works at all, ought to raise some very real questions about project evaluation and financial monitoring of nuclear projects. A British Fast Reactor of commercial size now appears to have been abandoned but the annual research and development allocations of £100 million plus continue. For how long? With what benefits? And for whom?

As an exposition of the manner in which nuclear power has developed its own peculiar brand of economics, which must be seen of course as a part only of its rationale, this book is informative and illuminating. Yet all books on nuclear power, without exception, ought to be read critically and with a certain degree of disbelief. Because this one does not carry a health warning, it needs to be consumed with at least as much care as its predecessors. □

Colin Sweet is Director of the Centre for Energy Studies at the Polytechnic of the South Bank, London.

The information man

Walter Gratzer

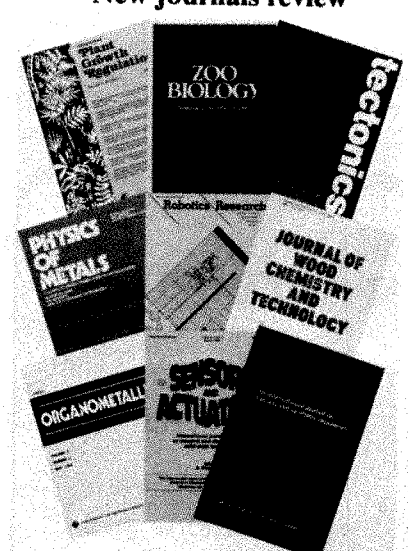
Essays of an Information Scientist, Volume 5.
By Eugene Garfield.
ISI Press, 3501 Market St, Philadelphia: 1983. Pp.848. $25 (North America), $28 (elsewhere).

"Ah, Mr Gibbon", the Duke of Gloucester is supposed to have observed, on being presented with the second volume of *The Decline and Fall of the Roman Empire*, "another damned, fat, square book. Always scribble, scribble, scribble, eh?". Likewise Eugene Garfield, whose business is the printed or processed word, and whose own output with this, his fifth volume of essays, culled from *Current Contents* and running to 848 pages, has probably by now exceeded Gibbon's life's work in the majesty of its bulk. It is true that, as he explains, in the essay entitled "How do you do it? Write all those essays, I mean", Dr Garfield has the advantage of Gibbon in that he marshals a staff of helpers who man his organization (the Institute for Scientific Information) and have titles such as Director of Communication, Manager of Bibliographic Research and Manager of Editorial Materials, not to mention two secretaries, called Sharon and Mary, their pens ever poised for dictation. All the same Dr Garfield's achievement is considerable, for he maintains a high level of readability, and from the well of statistics about science that he has sunk there gushes an inexhaustible stream of curious, interesting and sometimes outlandish information.

Dr Garfield has done more than invent *Current Contents* and the *Science Citation Index* to smooth the path of scientists: he has used the new information technology to generate a whole new human activity, which will undoubtedly become known (if it is not already) as *scientometry* or *scientometrics*. Although it had probably occurred to no one before Garfield had his first brainwave that anything of much consequence could be inferred from the references appended to every scientific publication, their function has undoubtedly undergone a surreptitious transformation. From being merely part of the *apparatus criticus*, designed to assist the reader to acquaint himself most easily with the background of the subject, references have acquired, with the emergence of "peer-review" and centralized funding, a ritual significance. They are now selected mainly to flatter, mollify or disarm. Why else, if not with a shifty eye to the impression that their bibliography might make on present or future members of a Study Section, do authors, as one of Dr Garfield's analyses has shown, cite by preference the work of their own compatriots at the expense of that from other countries? Conversely, scientists with undernourished egos are, as every editor knows, apt to make their dissatisfaction known if they believe their work to have received insufficient acknowledgement. As for autoreferencing (I cite Dorothy Parker), the advantage of self-praise is that it can be laid on thick and when it is most needed. Now that *Science Citation Index* ratings have even found a place in litigation, over such diverse matters as tenure or promotion and alleged maltreatment of laboratory animals, we disregard them, it seems, at our peril.

Like mercy then, citation is twice blessed (or three times if one takes account of ISI and Dr Garfield); it blesseth him that gives and him that takes. Moreover, Dr Garfield leaves us in no doubt that his league tables reflect merit in some sort. He rebuts the commonly heard argument that the best way to join that blessed host of the most cited authors — in Dr Garfield's terminology the *Nobel Class*, no less — one need only make some trifling improvement in an analytical colour reaction or devise a new buffer system for gel electrophoresis, by demonstrating that only a minority of the *Nobel Class* would be supplanted if their

New journals review



On 27 September *Nature* will publish the fourth annual review supplement devoted to science journals.

Criteria for inclusion of a journal in the 1984 issue are that:

(i) the first number appeared, or the journal was retitled, *between June 1982 and May 1983* (the second cut-off date allows at least three issues of a journal to have been published, the minimum number on which a reasonable judgement can be based);

(ii) it is published at least three times a year;

(iii) the main language used is English.

Publishers and learned societies are invited to send *four different issues of each suitable periodical, including the first and most recent numbers* (if from outside the United Kingdom, by air mail) to: The Review Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF, England. Subscription details, if possible for both 1984 and 1985, should be included.

single most cited paper were taken away. And yet, and yet . . . looking down the register of the *Nobel Class*, we see amongst the Nobel laureates and aspirants the names (I would have thought by common consent) of some of the choicest noodles in all of science, as well as some striking absences (not a Huxley in sight for example); presumably some of these operate in sparsely populated fields, or then again perhaps, as Chargaff has suggested, it is less important to be first than to be last. Garfield himself quotes a testy letter from an illustrious virologist, to the effect that his *Citation Classic* (another of Garfield's coinages) is one of his less significant accomplishments, being cited exclusively for the procedure that it contains for washing and suspending bentonite clay, which is used as a nuclease inhibitor.

All the same, whatever one's (wholly subjective) reservations, Dr Garfield's statistics afford a unique panorama of science in action, and many a diverting insight into the intimate habits of its practitioners. The refinements of authorship are particularly rewarding. We learn for example that with success comes a modicum of generosity: Nobel laureates, once the bacon is safely home, tend to yield priority to their co-workers in the listing of authors. An examination of papers published in the *Journal of Physiology*, which as a matter of editorial policy places authors' names in alphabetical order, reveals that Professors with names such as Zweig, or those who have graduate students called Aardvark, take their business elsewhere. Indeed the citation-watchers, such as Professor Derek de Solla Price, have, it appears, been much exercised about how to apportion credit for authorship: should the first author be entitled to store up more riches in heaven than the second or the twenty-second? Should one receive equal benison for a share in a thirty-author bone-crusher of the kind common in particle physics, as for a solo effort? The experts, you will be relieved to hear, are working on it.

The scope of Dr Garfield's writings transcends the work of ISI, however. When Charles Dickens received as a contribution to the literary magazine, of which he was editor, a set of poems with the title "Orient pearls at random strung" he sent it back with the scribbled comment, "too much string". There is, to be sure, a certain amount of string in Dr Garfield's latest anthology, but overall he gives excellent value. His organization has endowed prizes and sponsored local artists; he writes pleasantly about these enthusiasms, and his dissertations on such subjects as risk assessment, and most of all on medical matters, are at times quite transfixing. His book is worth the money just for his accounts of those three arguments against the existence of God, acne, halitosis and venereal trichonomiasis. One looks forward to more in the same vein anon. □

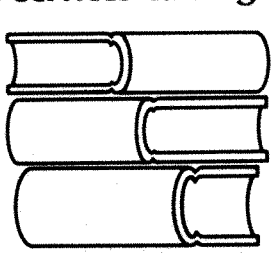
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Metals on the brain

Laszlo Magos

Neurobiology of the Trace Elements (in two volumes).
Edited by Ivor E. Dreosti and Richard M. Smith.
Humana: 1983. Vol.1 pp. 374, Vol.2 pp.320. Each volume $49.50 (US), $59.50 (elsewhere).

THE title of this work refers to trace elements, but it is not without significance that only one chapter in the two books deals with an element which is not metal or metalloid. The first volume is dedicated to *Trace Element Neurobiology and Deficiencies*, the second to *Neurotoxicology and Neuropharmacology*; neuropharmacology, however, is represented by a single contribution only.

Though there are many common targets for essential and toxic metals — for example catecholamine metabolism for copper and manganese, or hippocampus for zinc, lead and trimethyltin — market research probably indicated that interest in one volume would frequently exclude interest in the other. As evidence of this, both volumes have the same foreword, preface and conclusions. As a toxicologist I cannot agree with the assumption — I found the first volume more cohesive, comprehensive and altogether better reading. One has the impression that the editors, with experience in trace metal deficiencies, were better equipped to conduct the authors of the first volume to play in concert but did not influence the authors of the second to the same extent. Moreover the correction of inconsistencies in the chapter on manganese in Vol.2 (for example when absorption is described) would have greatly improved this contribution.

In the first volume there are two chapters on copper, three on zinc and single chapters covering iodine, iron, selenium and cobalamine. There is very little repetition when more than one chapter is dedicated to the same metal. The authors balance well biochemistry, pathology, clinical and experimental investigations, and amalgamate diverse knowledge effortlessly, putting the main emphasis on developmental neurobiology.

The second volume has an excellent chapter on mercury, but this account is rather narrow in scope being restricted to the effects of methylmercury on the fetal brain. The hiatus is filled by a contribution on the behavioural toxicology of heavy metals, which, in addition to covering behavioural effects, concisely describes the neurotoxicity of methylmercury, metallic mercury and lead, and at least mentions arsenic and organotins. Given the effect of trimethyltin on the hippocampus, however, a chapter on these compounds should have been included. The rest of Vol.2 contains two chapters on cadmium and three on lead, with manganese, aluminium and lithium each being represented by single chapters. Though the teratogenecity of zinc deficiency is described in the first volume in detail, and the protective effect of zinc against the teratogenecity of cadmium in the second volume, it is not mentioned that cadmium inhibites the placental transport of zinc. Nevertheless the defects of the second book are relatively minor and it mainly suffers from comparison with Vol. 1.

Researchers in fields related to the neurobiology or neurotoxicology of trace metals will probably find, as I did, the reading of this not unusually expensive work to be rewarding. Beyond the information supplied, the quality of paper is excellent, the typography is clear and pleasant, and references, complete with titles, are plentiful and are arranged in alphabetical order at the end of the chapters. Both volumes are supplied with good indexes. □

Laszlo Magos is in the Medical Research Council Toxicology Unit, Carshalton, Surrey.

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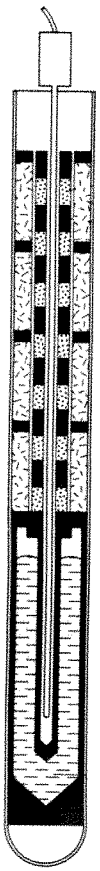
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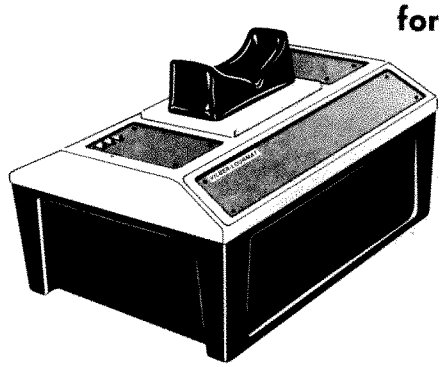
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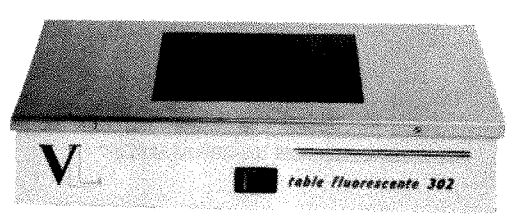
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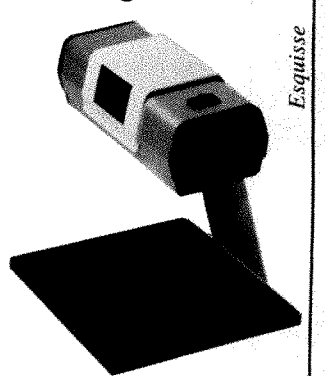
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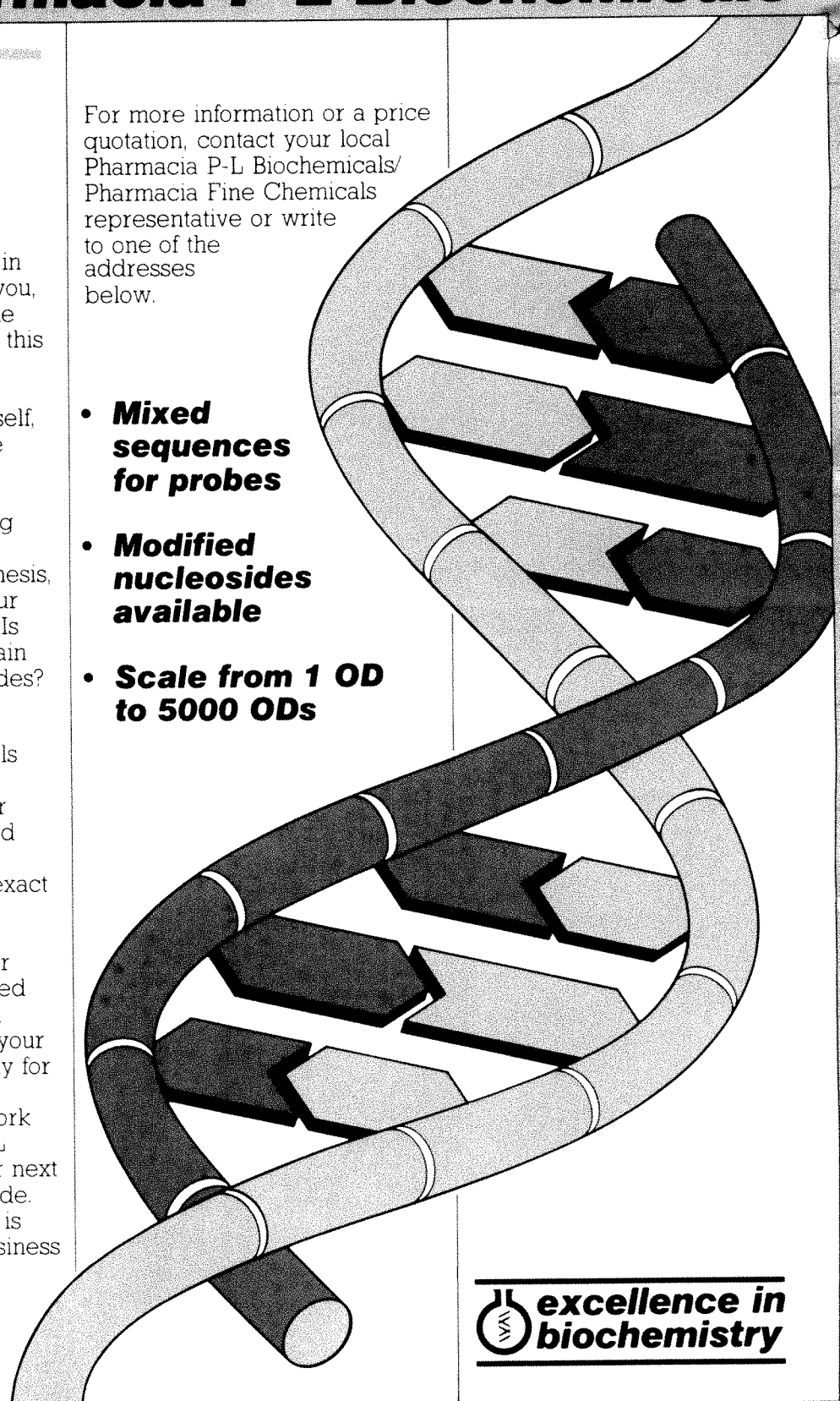
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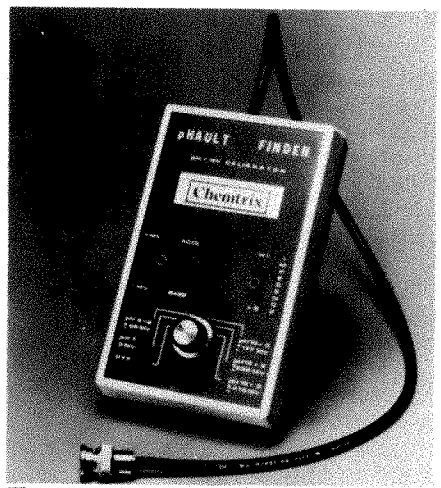
● Two new nonisotopic assays for monitoring thyroid function have been announced by Syva, the Syva Advance thyroxine assay and the Syva Advance T-3 uptake assay. Using fluorescence energy transfer immunoassay (FETI), the assays are designed to run on the Syva Advance system The thyroxine assay measures total (protein-bound and unbound) thyroxine in serum. The T-3 uptake assay measures human-serum binding capacity for thyroid hormone. The assays require only a small sample size (25 μl for thyroxine; 40 μl for T-3 uptake), and both assays use a simple two-point calibration.
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● The Gilson 401 dilutor is a single-syringe diluter that dispenses, pipettes and dilutes with very high accuracy and precision for liquid sample handling. In addition to the usual performances expected from an automatic and programmable dilutor, the Gilson 401 will perform sequential dilutions, aspirate up to 12 different samples in a single cycle, split an aspirated volume of reaget into up to 12 fractions and function in "reverse pipetting" mode to eliminate the risk of cross contamination. Programming is via a simple keypad controller and its microprocessor based ciruitry can store up to 90 programs for easy access. This dilutor may also be externally controlled from a computer.
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● A comprehensive reference list is available from Vector Laboratories covering 120 publications using the ABC immunoperoxidase staining system. In addition to light microscopy with polyclonal antibodies on parrafin sections, applications include staining of frozen sections, cytocentrifuge preparations, solid phase enzyme immunoassays, nitrocellulose blots, and pre- and post-embedding EM using monoclonal antibodies, lectins and biotinylated polynucleotide hybridizing probes.
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● The derivatizing reagent phenylisothiocyanate (PITC), the major reactant in the Edman degradation procedure for sequencing proteins and peptides, has been modified by Waters Associates to provide a new HPLC technique for amino acid analysis. Termed PICO-TAG, the new method offers 1 picomole detection in analysis of primary and secondary amino acids in only 15 minutes.
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These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.



The new pH/mV calibrator from Chemtrix.

● An extensive range of avidin conjugates for use in indirect immunoassays is available from Tago Inc. The avidin conjugates are now available with FITC, rhodamine, Texas Red, horseradish peroxides, alkaline phosphatase, or beta-galactosidase. Quality performance is confirmed by ELISA testing and histological microscopy. These new avidin conjugates are companion products to a wide line of biotinylated xenogenic affinity-islolated antibodies and $F(ab')_2$ fragments specific for human, mouse, rat, rabbit or pig Ig.
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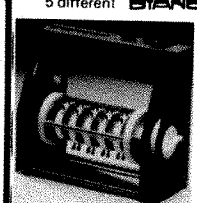
● The new Kratos MS 25 RFA mass spectrometer features new magnet technology, faster scanning capabilities and a higher mass range than previous models. The fast scanning (0.1s) magnet permits acquisition of high quality spectra from extremely narrow GC peaks, and the higher mass range of >3,000 AMU at 1 kV delivers high quality high mass spectra when used in conjunction with a fast atom bombardment source. Combined with the new DS 90 colour graphics data system, the MS 25 RFA offers levels of performance, previous available only using more expensive high resolution GC/MS systems.
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● The Chemtrix "pHault Finder" is a hand-held *p*H checker which can quickly and accurately test the calibration, temperature compensating circuits, input impedence and millivolt output of a *p*H meter. This unit stimulates the input from a *p*H electrode in *p*H units, 0 to 14 *p*H or mV units, +355 to −414 mV units. For each unit change in *p*H, the millivolt reading shifts ±59.1 mV units. Additionally, the automatic and manual temperature compensating circuits of a meter may be verified with the pHault Finder in 25°C steps between 0° and 100°C.
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● The VG Instruments PlasmaQuad is a powerful new system for elemental analysis combining an inductively coupled plasma with a high-resolution quadrupole mass spectrometer (ICP-MS). The ICP-MS technique gives cleaner spectra than other analytical methods for lower limits of detection and low noise levels.
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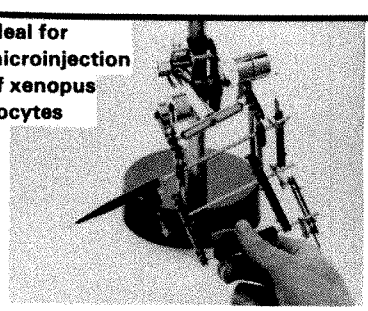
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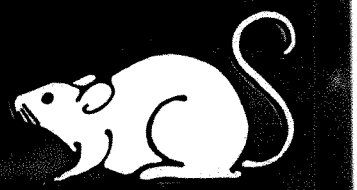
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MONOCLONALS

Bio-Rad's DEAE Affi-Gel® Blue has been used successfully to purify rabbit and human IgG from whole serum. Now it's been reported[1] that this same one-step chromatographic procedure can be used to purify mouse monoclonal antibodies directly from ascites fluid under mild conditions–pH 7.2. The resulting IgG fraction is free of protease and nuclease activity and is stable to long term storage. The authors include a chromatogram (Fig. 1) showing the separation of IgG_1. The procedure is also effective for IgG_2.

1. Bruck, C., Portetelle, D., Gilneur, C. and Bollan, A., *J. Immunol. Methods*, **53**, 313 (1982).

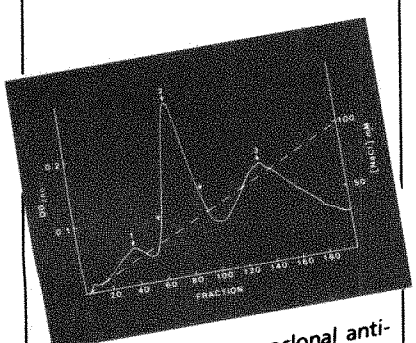


Fig. 1 Elution of monoclonal anti-urokinase antibodies (IgG_1) from a DEAE Affi-Gel Blue column.

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For details, contact:

BIO-RAD

Bio-Rad Laboratories–
Chemical Division
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(415) 234-4130

Circle No.02 on Reader Service Card.

● The new brain slice chamber from Medical Systems Corporation of Great Neck, New York features a single base unit and two interchangeable top units. The Haas top configuration utilizes semi-submersion techniques, providing good stability for intracellular recording and rapid exchange for perfusion fluids. The Zbicz top is designed to maintain viable slices of tissue submerged under a constant flow of perfusing liquid. A range of techniques can be used for evaluation of drug actions. Cell longevity of 10 or more hours is possible. A companion temperature controller is offered which functions with either top chamber and regulates temperature 30°–40° ± 0.2°C.
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● Dynatech Laboratories' AM572A multi-purpose Autowash washer/aspirator can deliver wash fluid and aspirate a complete 96-well MicroElisa plate in 60–90 seconds, depending on the specific test requirements. The machine is therefore suitable for the smaller laboratory with a limited through-put of Miro-Elisa tests as well as being capable of handling the demands of the large-scale user. It can also be used in certain RIA and blood-transfusion applications. A simple but accurate depth adjustment on the wash/aspirate manifold makes the AM52A Autowash particularly suited to working with tissue culture monolayers.
Circle No. 109 on Reader Service Card.

● A new test kit for the determination of angiotensin converting enzyme (ACE) in serum is available from Boehringer Mannheim. The colorimetric assay is based on the ability of ACE to convert hippuryl-glycyl-glycine into glycyl-glycine which is converted to trinitrophenyl-glycyl-glycine by trinitrobenzylsulphonate. The absorbance of trinitrophenyl-glycyl-glycine is measured at 420 nm. The test requires a sample of only 10 μl.
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● Very low levels of NADH and NADPH can be detected using the new LKB bioluminescent assay. The reagents use bacterial luciferase coupled to a specific oxidoreductase enzyme. There are therefore two reagents, one for NADH, the other for NADPH. These reagents give a vastly improved (1,000-fold) sensitivity compared with spectrophotometric assay for NADH, NADPH and associated enzyme assays. An application paper is available from LKB.
Circle No. 111 on Reader Service Card.

● New from Cruachem, ColorTAG monomers can be used during the synthesis of a mixed DNA probe to determine the success or failure of a mixed addition. The deprotection effluent from each of the monomers is a distinctive colour and so the actual nucleotide ratio at a degenerate site can be determined by visible spectroscopy. This provides confirmation that all possible sequences are indeed present in a mixed probe.
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● For decontamination of laboratory glassware used with radioactive solutions, ICN Radiochemicals is marketing Dekasol. After a 24-hour soak at room temperature in 5% Dekasol and distilled water, residual activity for carbon-14, tritium and phosphorus-32 is usually 0.2% of the original activity. For more stubborn radioactive substrates, such as those containing iron-49 and iodine-131, soaking in a 20% solution of Dekasol at 50°C for 2 hours provides effective decontamination.
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● Zetabind is a charge-modified membrane matrix for DNA, RNA and protein transfer applications. Manufactured by AMF Cunuo, Zetabind is composed of charge modified nylon 66 which results in an electrostatic adsorption of anionic macromolecules. Binding capacities of 480 $\mu g\ g^{-1}$ have been reported and this highly efficient binding permits re-use of transfers for re-probing. Zetabind, unlike DBM and DPT papers, does not require chemical activation and can be stored indefinitely at room temperature. Efficient binding of anionic macromolecules in low salt buffers permits electrophoretic transfers from gels. It also provides enhanced binding for capillary blots.
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Frozen serum panels from NCI

A VARIETY of serum components (including peptide hormones, viral antigens, isoenzymes, glycoproteins, antibodies, immune complexes, tumour-associated antigens, carbohydrates, phospholipids and nucleotides) have been reported to be useful in cancer diagnosis and/or in monitoring cancer treatment or recurrence. The US National Cancer Institute is interested in evaluating serum assays that are potentially useful in the diagnosis of cancer. Coded panels composed of 1 ml aliquots of pretreatment frozen sera from patients with various neoplasms, from benign disease patients and from healthy controls are available to investigators to evaluate assays in which preliminary results indicate the ability to discriminate between cancer patients and controls. Promising results may form the basis for a subsequent grant application. Preliminary data documenting a useful test must be submitted and should include: a brief description of the assay, results of patients with cancer, results of patients with non-malignant disease, results in healthy control subjects and reprints of published work, if available. Request for a coded serum panel should be sent to: Diagnosis Serum Panels, Project Officer, NCI-Serum Bank, Diagnosis Branch, Westwood Bldg., Room 10A10, 5333 Westbard Avenue, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205. □

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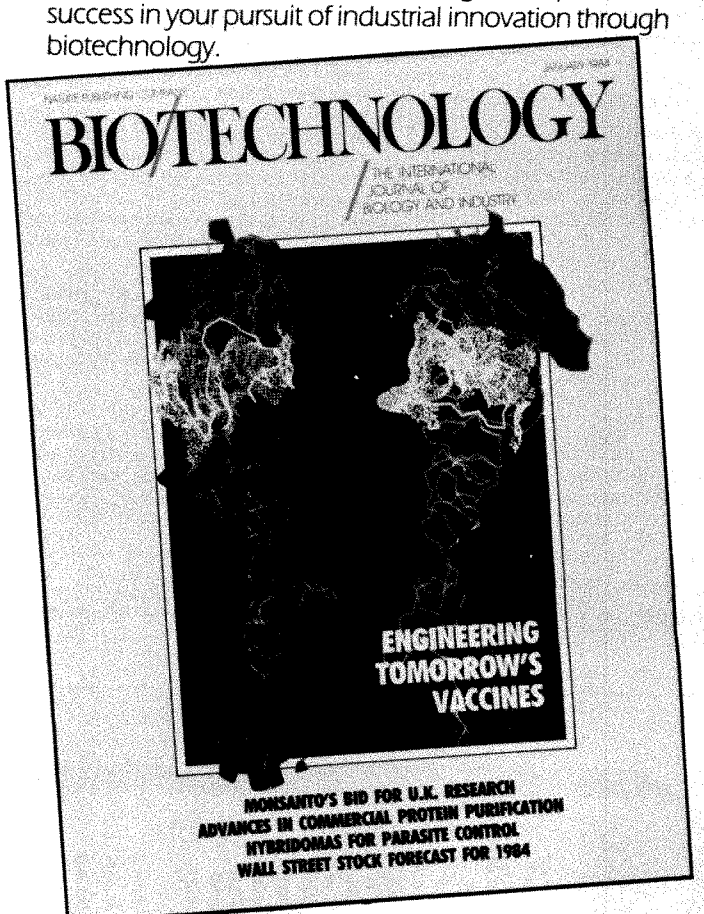
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UNIVERSITY OF OTAGO
Dunedin, New Zealand

CHAIR OF MATHEMATICS

The University Council invites applications for appointment to the Chair of Mathematics at present occupied by Professor D B Sawyer who will retire at the end of 1984.

Applicants should possess a substantial research record and good administrative and teaching ability. Candidates in any area of specialisation will be considered but preference will be given to candidates whose interests are centred in the main body of Mathematics.

Professional salaries at present provide for a salary within the range of NZ$41937 to NZ$52182 per annum. In addition, a cost of living allowance of NZ$417 per annum will be paid.

Further particulars are available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, University of Otago, PO Box 56, Dunedin, New Zealand.

Applications close on 31 August 1984. (2206A)

BILLERICAY BURNS RESEARCH TRUST

SENIOR RESEARCH FELLOW

Applications are invited for a Senior Research Fellow for a project in burns research. Considerable experience in independent research will be required. The Fellow will be expected to pursue not only his own project but also to give advice and guidance on their research projects to surgical staff at this Regional Centre for Plastic Surgery and Burns.

The Fellow's research project might be in the structural, biochemical, pharmacological, physiological or experimental pathological fields.

It is envisaged that the Fellow would be of a status such as would enable the Trust to seek for him or her an honorary university appointment in one of the University of London Medical Schools.

Further details may be obtained from: A F Wallace, FRCS, NE Thames Regional Plastic Surgery Centre, St Andrew's Hospital, Billericay, Essex. (2175)A

BRISTOL POLYTECHNIC
SCIENCE DEPARTMENT

RESEARCH ASSISTANT — Ref No R/34

Applications are invited from honours graduates in Biological Science or from candidates completing their degrees this Summer for the post of Research Assistant to Dr A Vivian, Reader in Biotechnology, to investigate the Genetics of Host specificity in Pseudomonas pisi.

This is a 3 year project funded by the Agricultural and Food Research Council and the successful candidate would be expected to register for a higher degree.

Salary Scale: £5,415 — £6,123 per annum.

For further details and an application form, to be returned by 11 June 1984, please contact the Personnel Office, Bristol Polytechnic, Coldharbour Lane, Frenchay, Bristol or ring Bristol 656261, ext 216 or 217.

Please quote Reference Number R/34 in all communications. (2180)A

THE FLINDERS UNIVERSITY OF SOUTH AUSTRALIA
SCHOOL OF PHYSICAL SCIENCES
RESEARCH ASSOCIATE

Applications are invited for a position as Research Associate in the School of Physical Sciences. This position is supported by the Department of Resources and Energy for work on the development of new heterogeneous catalysts for the production of hydrocarbon fuel. Applicants should have a PhD and relevant research experience in catalysis or solid state chemistry.

The appointment will be made in the salary range $A22,212-$A26,536 (under review) according to qualifications and experience and will be until June 1986 in the first instance. Further information may be obtained from Professor B G Baker, Tel (08) 275-2288.

Applications, including full personal details, details of academic record, experience and publications, and the names and addresses of at least two referees, should be sent to the Registrar, The Flinders University of South Australia, Bedford Park, South Australia 5042, by 30 June 1984. Applicants are requested to ask their referees to forward their reports direct to the Registrar before the closing date. (2202)A

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(2173)A

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Salary commensurate with training and experience. Send curriculum vitae and three references to: F Meyskens, Jr, University of Arizona Cancer Center, 1501 N Campbell Avenue, Tucson, AZ 85724.

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required to work on a project concerned with the endocrine regulation of nutrient supply and utilisation in sheep. The position is funded by the Agriculture and Food Research Council for a fixed period of two years.

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Apply, quoting Ref R23A, for application form and further details to Personnel Office, University of Reading, Whiteknights, Reading RG6 2AH. (2186)A

UNIVERSITY OF EDINBURGH

FACULTY OF MEDICINE

CHAIR OF PHARMACOLOGY

Applicatons are invited for the Chair of Pharmacology in the Faculty of Medicine in the University of Edinburgh.

The Professor of Pharmacology will be expected to provide academic leadership as Head of Department, to promote and conduct research and to participate in undergraduate and post graduate teaching.

The salary will be at the pre-clinical professorial level (minimum £17,910 per annum).

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, EH8 9YL, with whom applications (15 copies), including curriculum vitae and the names and addresses of three referees should be lodged not later than 31st July, 1984.

Please quote reference no 22/84. (2165)A

Commonwealth of Australia
Department of Defence
Canberra

SENIOR DEFENCE OFFICER

(Nuclear Development Analysis)

$A27952-29048

The Australian Department of Defence invites applications from suitably qualified men and women for the position of Senior Defence Officer in the Joint Intelligence Organisation located in Canberra.

Canberra, the national capital, with a population of 220,000, is a modern city situated in pastoral surroundings approximately 200 kilometers inland from the major port of Sydney. It enjoys a temperate climate and is within driving distance of seasonal summer and winter recreational areas. The city offers excellent community facilities particularly for those with a young family.

The Joint Intelligence Organisation is responsible for the preparation of reports and strategic estimates, both of a long term and current nature, on a wide range of subjects of a defence interest, mainly in the Asia-Pacific region.

The successful applicant for this position will carry out documentary research and prepare reports on foreign nuclear research, development and production.

Qualifications in science or engineering at a tertiary level and several years experience in the nuclear industry or research fields are desirable.

Conditions of service include four weeks annual leave with a bonus payment, cumulative paid sick leave, maternity leave after a qualifying period, three months long service leave after ten years continuing service and a comprehensive superannuation scheme. Fares and removal expenses to take up duty and settling-in allowances are payable in some circumstances.

Applications giving full details of qualifications, relevant experience and employment history, together with recent references and telephone numbers of referees, should be forwarded to: Mr. R. Rankin, Personnel Services, Third Floor, Australia House, Strand, London WC2B 4LA (Tel: 01-438 8829) by 29th June, 1984.

(2229)A

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WELLCOME BIOTECHNOLOGY LIMITED, FMD DIVISION, PIRBRIGHT

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Applicants should be highly motivated, educated to degree or HTEC level and have the practical experience to provide full technical expertise required to general full length DNA clones of Foot and Mouth Disease Virus RNA. To obtain infectious virus from such clones and to perform site directed mutagenesis.

The post will attract a competitive salary and excellent benefits including 5 weeks holiday, life assurance, sick pay and pension scheme.

Please write with full details to: Mr. K. Tollett, Wellcome Biotechnology Limited, FMD Division, Ash Road, Pirbright, Woking, Surrey GU24 0NQ. (1905)A

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DIVISION OF MATHEMATICS AND STATISTICS CANBERRA ACT

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Mathematics and Statistics is a member of the Institute of Physical Sciences.

The Division of Mathematics and Statistics has a staff of 97, including 75 professional scientists. The Division's Headquarters are at Canberra, and there are Regional Offices located at Melbourne, Sydney, Adelaide, Brisbane and Perth. Scientists of the Division provide advice on the statistical, mathematical and numerical aspects of research projects undertaken by scientists in other CSIRO Divisions, as well as conducting basic research in statistic and mathematics.

The Division has a program in Signal Analysis and wishes to consolidate and expand this program's work in Image Analysis. The appointee will be required to direct the Division's research and consulting in this area. The appointee will be located in Sydney but may be required to visit other cities in Australia to initiate and direct collaborative work with scientists in other Divisions in CSIRO.

Applicants should have a PhD degree or equivalent qualifications in a relevant branch of statistics together with research and consulting experience in at least one of the fields of geometrical probability, mathematical morphology or stereology.

The position is available for a term of five years and Australian Government superannuation benefits are available.

APPLICATIONS: Stating full personal and professional details, the names of at least two referees and quoting reference No A3591, should be directed to:

The Chief
CSIRO Division of Mathematics and Statistics
GPO Box 1965
CANBERRA ACT 2601
AUSTRALIA

By June 29, 1984.

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(W1021)A

SENIOR ANIMAL SCIENTIST

The Sub Humid Zone Programme of the International Livestock Centre for Africa, Kaduna, Nigeria, seeks a SENIOR ANIMAL SCIENTIST with qualifications and experience in cattle production but who should also have a strong interest in small ruminants and methods of pasture management. The programmes work is focussed on mixed farming systems and the interface between pastoral and agricultural communities in the sub-humid zone of West Africa. There is a strong emphasis on the use of legumes for forage improvement in these systems.

We are seeking an individual who can work as part of a small interdisciplinary team with a farming systems research approach and who is able to develop innovative approaches to research on small holder and pastoral animal production systems. An interest between nutrition health and production is essential. West African experience and French language are highly desirable.

Salary and related benefits are negotiable in line with comparable International Organisations.

Applications should airmail personal history and names of three referees to:

The Director General,
ILCA, P.O. Box 5689 — Addis Ababa, Ethiopia

to reach him before July 31, 1984. (W1023)A

UNIVERSITY OF EDINBURGH

DEPARTMENT OF PHYSICS

Applications are invited for the post of

UNIVERSITY DEMONSTRATOR

in the Department of Physics from 1st October 1984 for a period of 3 years. The appointee will be expected to contribute to the Department's teaching and to join in the work of one of the research groups; preferably that of the experimental condensed matter group.

The appointee will probably already have a PhD degree and the salary will be in the range £6,310 — £9,425 dependent upon age and experience, together with normal pension arrangements.

Applications, including the names of two referees, should be submitted to Professor R A Cowley, University of Edinburgh, Department of Physics, Mayfield Road, Edinburgh EH9 3JZ, from whom further particulars can be obtained.

Please quote reference No 1135. (2194)A

UNIVERSITY OF READING

WELCOME TRUST LECTURESHIP

DEPARTMENT OF PHYSIOLOGY & BIOCHEMISTRY

Applicants should have research experience in the physiology of the fetus or newborn, and an interest in the neurophysiology of development would be an advantage. The appointment is from 1 October 1984 for three years and may be renewed for a further two years subject to satisfactory review.

Applicants should not be more than 35 years old or holders of a tenured post. Current Lecturer's salary £7,190 to £14,125 pa.

Further particulars and application forms (2 copies) are available from the Personnel Officer, University of Reading, Whiteknights, Reading, RG6 2AH, Tel (0734) 875123 ext 220. Please quote Ref AC 8413. Closing date 18 June 1984. (2157)A

UNIVERSITY OF NOTTINGHAM

LECTURER IN ZOOLOGY

Applications are invited for the post of Lecturer in Zoology which will become vacant on 1st September 1984. Candidates will be expected to have research experience in behavioural and/or applied fields of Insect Biology.

Salary will be within the range £7,190 — £14,125 per annum.

Application forms and further particulars, returnable not later than 18 June 1984, may be obtained from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Ref no 948. (2178)A

UNIVERSITY OF LIVERPOOL

DEPARTMENTS OF IMMUNOLOGY AND BIOCHEMISTRY

Applications are invited for the post of

SENIOR RESEARCH ASSISTANT

in the Department of Biochemistry to work on an investigation into gene cloning of human oncotrophoblast antigens defined by monoclonal antibodies. This work has important implications for our understanding of materno-foetal interactions as well as the process of oncogenesis. This is a post-doctoral postion for which experience in recombinant DNA techniques would be a strong advantage.

Initial salary within the range £7,190 — £8,080 per annum.

Applications, together with the names of three referees, should be received not later than 25th June, 1984, by the Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/529/N. (2176)A

UNIVERSITY OF GLASGOW

DEPARTMENTS OF BIOCHEMISTRY AND MEDICAL CARDIOLOGY

GRADUATE RESEARCH ASSISTANT

A vacancy exists for a graduate with a good degree in Biochemistry or Molecular Biology to work on a project to investigate control of energy metabolism in the heart. Salary will be on Range 1B of the scales for Research and Analogous Staff (£6,310—7,630).

Applicants should send their CV and the names of two academic referees to Dr Jocelyn Dow, Department of Biochemistry, University of Glasgow, Glasgow, G12 8QQ, to arrive not later than 8th June, 1984.

In reply please quote Ref No 5264M. (2167)A

SOUTH MANCHESTER HEALTH AUTHORITY

CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE

PATERSON LABORATORIES

RESEARCH TECHNICIAN/ SENIOR RESEARCH TECHNICIAN

For work in a Histopathology Laboratory providing a service to biological research units.

Qualifications: HNC in Medical Laboratory Sciences (Histopathology) or equivalent experience.

Salary: £5,491 — £7,747 or £7,997 — £9,507 pa according to age, qualifications and experience.

Applications to Laboratory Administrator, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Wilmslow Road, Withington, Manchester M20 9BX quoting reference PL/84/10 and enclosing SAE. (2174)A

UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT DALLAS

DEPARTMENT OF RADIOLOGY

POSTDOCTORAL RESEARCH
NMR APPLIED TO TISSUE METABOLISM

Two positions are available to pursue research in physiology and metabolism using the non-invasive method of high resolution nuclear magnetic resonance. The laboratory is currently investigating several aspects of cardiac and brain metabolism using NMR techniques. Research projects in both basic studies and in clinical applications are available. The facilities of the NMR Laboratory are extensive and state-of-the-art and include a one meter bore system under development for clinical use. The laboratory also has an excellent staff to support various aspects of the research. Candidates should have training in biochemistry or physiology. Additional experience in physical and analytical chemical methods is desirable but not essential.

Please send a résumé and three letters of reference to: Ray L. Nunnally, PhD, Director of Biomedical Magnetic Resonance, Department of Radiology, University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235.

Equal Opportunity University.
(NW530)A

TRUDEAU INSTITUTE

A Postdoctoral research position in infectious disease

is currently available to study interactions between several intracellular microbial pathogens and the cellular defenses in both normal and immunosuppressed animals. Current research is directed towards understanding cellular interactions responsible for the modulation of delayed-type hypersensitivity and acquired antimicrobial resistance during the development of chronic systemic disease in inbred mice. Candidates should have a strong background in infection-immunity or in immunology.

The Trudeau Institute is a private, not-for-profit basic medical research institution situated on the shores of Lower Saranac Lake, about a mile from the village of Saranac Lake and 10 miles from Lake Placid, NY. On-campus housing is available. Salary is at appropriate NIH levels.

Send curriculum vitae and names of three references to: Dr Frank M. Collins, Trudeau Institute, P.O. Box 59 Saranac Lake, N.Y. 12983. An Equal Opportunity Employer.
(NW525)A

ADVANCED DRUG DELIVERY RESEARCH

Horsham, West Sussex

In line with the policy of Ciba-Geigy Pharmaceuticals to lead the field in technological innovation, a major new research unit dedicated to the discovery and exploitation of new concepts for drug delivery has been established in Horsham. This unit will provide exceptional opportunities for imaginative and highly motivated scientists to carry out original research in a stimulating and challenging environment in collaboration with scientists from a wide range of scientific disciplines.

Applications are now invited for the following positions:

Senior Section Leader in Colloid/Polymer Science

To lead and participate in the creation of a section of the unit responsible for the design and characterisation of novel colloidal drug delivery systems. The applicant should have demonstrated outstanding ability in colloid/polymer science or in a related field. Ref ACP 1

Research Scientists in Colloid/Polymer Science

Positions are available for a scientist having a PhD with, preferably, at least two years post-doctoral experience, and for graduates with a strong background in colloid and surface chemistry/polymer science or a related field. Ref. ACP 2

Research Biologist/Physiologist

To participate in a Section of the Unit responsible for examining the biological aspects of colloidal drug delivery systems. This post offers an unique opportunity to a graduate having a particularly strong background in mammalian biology/physiology to contribute to a multidisciplinary research unit. Ref ACP 3

The salary and benefits package for these positions is very attractive and will include relocation expenses where appropriate.

Applicants for these positions should send a full c.v. and an outline of their research and career expectations, quoting reference, to Miss S.J. Byrne, Personnel Officer, Ciba-Geigy Pharmaceuticals, Wimblehurst Road, Horsham, West Sussex, RH12 4AB.
(2158)A

CIBA–GEIGY

BIOCHEMISTRY: Two tenure-track assistant professors effective from 1 September 1984. Postdoctoral experience and a commitment to teaching and research required. Research areas open, but preferences to neurochemistry and molecular/cell biology. Application deadline: 1 July 1984. Send résumé, research plans, summary of teaching experience, and at least three letters of recommendation to: Chairman, Departmental Affairs Committee, Department of Biochemistry, Emory University, Atlanta, Ga 30322. An Equal Opportunity/Affirmative Action Employer. (NW569)A

MARIE CURIE MEMORIAL FOUNDATION RESEARCH INSTITUTE

Two Post-doctoral positions in Molecular Biology. The first will involve the application of recombinant DNA technology to the study of cellular gene activation by transforming proteins.

The second will investigate the role of the transformation – associated protein p53 in cell proliferation and transformation. Eukaryotic vectors expressing p53 and a variety of mutants are available for this work.

These three-year appointments are available now and salary will be on the Lecturer Scale.

Apply with C.V. and the names of two referees to Dr. G. Currie, Marie Curi Research Institute, The Chart, Oxted, Surrey (088388-3055).
(2163)A

Instrumentation Laboratory's Expansion In Diagnostic Research

Allied, a 10-billion dollar diversified company, has made a major commitment to growth in Human Medical Diagnostics.

This is a new venture focusing on research and development of diagnostic products for human diseases.

The following positions are currently available at Instrumentation Laboratory, Allied's diagnostic company, located in Lexington, Massachusetts.

Scientists

Biochemist

Evaluate biotechnologies having application in in-vitro diagnostic tests including immunodiagnostics, monoclonal antibodies and DNA probes. Design or develop diagnostic tests using technology available within IL or at other Allied divisions. Guide designs through production phase. MS biochemistry with 5-10 years' experience in diagnostics or biotechnology. Knowledge of immunology, monoclonal antibody technology. Job code BCH.

Senior Scientist

We seek an individual with 2 years' minimum hands-on experience operating a florescence activated cell sorter. Background in cell surface immunoflorescence, cell cycle analysis and DNA staining techniques is necessary. Experience screening and sorting hybridomas would be desirable. Responsible for maintenance and calibration of the equipment for own project and that of other investigators as well. MS/Ph.D required. Job code SSF.

Senior Scientist

We seek a Ph.D level scientist to research biological aspects of lymphokines. Requires broadbased experience including T-cell hybridoma methodology. Minimum 2 years' postdoctoral experience. Should include the isolation, purification and bioassays for lymphokines. Particular interest in TCGF (IL-2) and INF, but not restricted to them. Job code SCL.

Principal Scientist

Position involves reagent formulation, stabilization, and optimization. Ph.D. in Chemistry with 6-8 years' experience required. Simplex optimization methodology background a plus. Job code PLS.

IL offers an excellent salary and benefits package. Please forward resume including salary requirements and specifying job code for the position you are interested in to:
Mr. Paul A. Smith, Jr.
Dept. N1
Instrumentation Laboratory
113 Hartwell Avenue
Lexington, MA 02173

We are an equal opportunity employer

ALLIED Instrumentation Laboratory

(NW557)A

UMIST/ DARESBURY LABORATORY
SYNCHROTRON RADIATION — POST DOCTORAL POST IN X-RAY SPECTROSCOPY

Applications are invited for a Research Assistant on a project involving the application of X-ray Spectroscopy to the determination of the structure of copper containing enzymes involved in the biosynthesis and metabolism of biogenic amines. The position is tenable from 1st October 1984 and is for a period of three years. He/she is expected to participate in the further development of the EXAFS technique and particularly in the analysis of the near edge fine structure from which detailed three dimensional information around the active sites of the enzymes can be obtained.

Applicants are expected to hold a PhD in Physics, Chemistry or a biological science. Experience with the application of physical methods to biological structure determination would be an advantage.

Commencing salary; £7,630 p.a. on range IA. (under review).

Applications, quoting reference CH/ 78/ AI, including a curriculum vitae and names of two referees should be sent to either Dr N J Blackburn, Chemistry Department, UMIST, PO Box 88, Manchester M60 1QD or Dr S S Hasnain, Daresbury Laboratory, Warrington WA4 4AD. The closing date is 22 June, 1984.

(2190)A

UNIVERSITY OF ABERDEEN
DEPARTMENT OF MICROBIOLOGY
TEMPORARY LECTURESHIP

The Department of Microbiology invites applications for a temporary Lectureship for one year from October 1984. The post arises from the award of a Nuffield Research Fellowship to Dr I R Booth, and the successful applicant will be expected to assist with teaching in microbial physiology, genetics and molecular biology. There will be the opportunity also to carry through a research programme.

Salary within the range £7,190 to £8,530 per annum on Lecturers salary scale (under review).

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 15 June 1984.

(2170)A

UNIVERSITY OF NEWCASTLE UPON TYNE
CHAIR OF SOIL SCIENCE

Applications are invited for the Chair of Soil Science which will become vacant through retirement on 30 September 1984. The post is open to suitably qualified candidates in the field of Soil Science; some preference will be given to those with interests in Soil Chemistry. The appointment will carry the headship of the Department of Soil Science for an initial period of five years.

The appointment will be effective from October 1984 or as soon as possible thereafter. Salary will be at an appropriate point on the range for professional staff which commences at £17,275.

Further particulars may be obtained from the Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU, with whom applications (15 copies) giving the names of not more than three referees must be lodged not later than 6 July 1984. Candidates from outside the British Isles may submit one copy only.

(2198)A

MONASH UNIVERSITY
Melbourne, Australia
DEPARTMENT OF BIOCHEMISTRY
FIXED-TERM LECTURER (3-5 YEARS)

Postdoctoral graduate for teaching medical or science students and participation in research in areas of (1) cloning and gene expression in yeast; (2) human interferon biology, as well as genes and expression; (3) yeast mitochondril ATP synthetase complex; (4) monoclonal antibodies and protein chemistry. Enquiries to Professor A W Linnane.

Salary: $A24,353 — $A31,994 pa. Applications including Ref no 30212A, curriculum vitae and 3 referees to the Registrar, Monash University, Clayton, Vic 3168, Australia, with a copy to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, by 9/7/84, from whom Conditions of Appointment are available.

An equal opportunity employer.

(2068)A

UNIVERSITY OF EDINBURGH
FACULTY OF MEDICINE
LECTURESHIP IN PHYSIOLOGY

Applicants must have a good Honours Degree in Physiology and at least three years' research experience.

Salary will be at an appropriate point on the scale £7,190—£14,125. USS Superannuation.

Applications (8 copies) including a full curriculum vitae and the names and addresses of three referees should be sent to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, EH8 9YL by 25th June, 1984, from whom further particulars can be obtained.

Please quote reference 1134.

(2166)A

La forte expansion de notre

LABORATOIRE PHARMACEUTIQUE

et son rayonnement à l'étranger
nous permettent la création d'un nouveau

CENTRE DE RECHERCHE,

A cet effet, nous souhaitons rencontrer son futur

DIRECTEUR

Il participera au choix de son implantation géographique et sera chargé de son aménagement puis de sa direction.
Scientifique de haut niveau, tourné vers la pharmacologie, il sera suffisamment éclectique pour ANIMER et COORDONNER des équipes de chercheurs formées de chimistes et de pharmacologues innovant dans divers axes thérapeutiques.
La maîtrise de la langue française est nécessaire.

Prière d'envoyer lettre manuscrite en français, C.V. et photo, en précisant sur l'enveloppe la référence 6760 à Emplois et Carrières, 30 rue Vernet, 75008 Paris - France.

(2196)A

The East of Scotland College of Agriculture
The Edinburgh School of Agriculture

Head of Animal Production Advisory and Development and Deputy Head of Animals Division (Ref: 226/ N)

Applications are invited for the above senior post which has substantial responsibilities for the management of Research, Development and Specialist Advisory Work in Animal Production, Animal Production Systems and in the application of new techniques in Animal Science to farming practice.
Candidates should possess a good degree in Agriculture, Animal Production or Animal Science and considerable post-graduate experience in on or more aspects of the work.
The appointment will be on the Scottish Agricultural College's Grade I Level for which the current salary scale is £13,345-£17,748 per annum. The conditions of service are those appropriate to appointments at a similar level in the Civil Service.
Further particulars may be obtained from The Secretary, The East of Scotland College of Agriculture, The Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG to whom applications in writing, including the names and addresses of three referees, should be submitted not later than 30th June, 1984. Reference 226/ N, should be quoted in all communications.

(2159)A

RESEARCH ASSOCIATE
Post Doctoral

Cell biology or biochemistry. Basic studies on intracellular peptide processing, glycosylation, transport and secretion. 2-3 year appointments. Salary $20,000.

David V. Cohn, PhD Departments of Oral Biology and Biochemistry, Health Sciences Center, University of Louisville, Louisville, KY 40292. USA (502) 588-7507.

(NW543)A

VISITING RESEARCH Assistant Professor/Post doctoral non-tenure track position available Sept 1, 1984 for Molecular Biologist interested in working on cell-specific gene expression in Caenorhabditis elegans. Individual must have a PhD and expertise in DNA cloning and sequencing. Salary $18,000-$20,000 commensurate with experience and qualifications. Minimal teaching is required, full benefits, renewable yearly up to five years. Send résumé and 3 letters of recommendation to Dr Michael Klass, Biology Department, University of Houston, Houston, Texas 77004. (NW551)A

Cetus Corporation and Cetus Madison Corporation, leaders in the field of biotechnology, are currently seeking candidates for the following positions:

FERMENTATION MICROBIOLOGIST/ ENGINEER #84078

Successful candidates will have an M.S./Ph.D. and have experience in small and large scale fermentation. The position requires self-motivated individuals who are willing to be involved in bridging the gap between laboratory advances and commercial production.

Respond to this position to:

Personnel Dept., Cetus Madison Corp.
8520 University Green
Middleton, WI 53562

SR. SCIENTIST/SCIENTIST #83159

Cetus Corporation is currently seeking qualified Ph.D.'s for our Molecular Cloning Department. Candidates should have a minimum of three years' postdoctoral experience in the area of gene expression in mammalian cells and the construction of expression vectors.

ASSOCIATE SCIENTIST #84079

Candidates should have a Ph.D./M.D. with background in experimental regulation of human or murine IgE production.

RESEARCH ASSOCIATE/RESEARCH ASSISTANT (2) #83158/83106

These positions assist in research directed towards the isolation and characterization of mammalian genes and their products. Requires M.S. or B.S. and 3+ years of experience in molecular biology. Experience in mRNA isolation, characterization and cDNA cloning desirable.

RESEARCH ASSOCIATES (2) #83010

Candidates will assist in maintenance and operation of amino acid analyzer & sequencer, purification and biochemical characterization of native & cloned human proteins.

Successful candidates should have B.S./M.S. in Biology or Biochemistry with a minimum of 2 years' experience in protein chemistry especially in the areas of high and low pressure liquid chromatography, protein assays, gel electrophoresis and isoelectric focusing.

This is an opportunity to join a dynamic company that encourages professional growth.

Send resume or apply in person to:

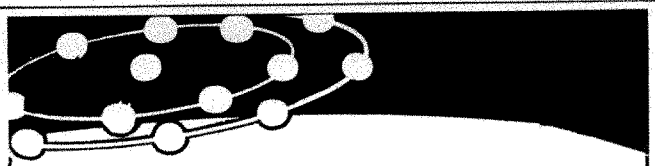
Cetus Corporation
Job # _____
1400 Fifty-third Street
Emeryville, CA 94608



(NW559)A

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The European Molecular Biology Laboratory, a research institute situated in Heidelberg, West Germany, invites applicants for the following vacancies at the Laboratory's Outstation at the Deutsches Elektronen-synchrotron (DESY) site in Hamburg

APPLICATIONS PROGRAMMER

The successful candidate will help the scientists at the Outstation to design, develop and maintain data acquisition and application programmes for the synchrotron radiation experiments at the Outstation which include protein crystallography, time resolved scattering and X-ray spectroscopy (EXAFS) on biological systems.

He/she will also be expected to take part in the daily operations of the computers at the Outstation: VAX 11/750, PDP 11/45, PDP 11/24. Computer network access is used to maintain collaboration with other groups. Since a large number of projects are carried out by visiting workers, an ability to interact with people is indispensable.

An interest in applying computer technology to scientific problems is indispensable and previous programming experience in a scientific environment will be an asset. The ideal candidate would have at least 3 - 5 years experience using one or more high level languages (FORTRAN, PASCAL). Knowledge of assembler for PDP 11 or VAX is an advantage.

The net salary offered will be around DM 3900, - monthly. Certain allowances are payable in addition, depending on personal circumstances.
An initial contract of 3 years' duration, which can be renewed, will be offered.

Please write briefly for an application form quoting reference N°84/23 to:

EMBL

Personnel Section,
Postfach 10.2209,
D-6900 Heidelberg

(W999)A

CSIRO
AUSTRALIA
STATISTICIAN (STATISTICAL ECOLOGY)
$A25,342-$A37,274
DIVISION OF MATHEMATICS AND STATISTICS
CANBERRA ACT

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Mathematics and Statistics is a member of the Institute of Physical Sciences.

The Division of Mathematics and Statistics has a staff of 97, including 75 professional scientists. The Division's Headquarters is at Canberra, and there are Regional Offices located at Melbourne, Sydney, Adelaide, Brisbane and Perth. Scientists of the Division provide advice on the statistical, mathematical and numerical aspects of research projects undertaken by scientists in other CSIRO Divisions, as well as conducting basic research in statistic and mathematics. The current statistical programs of the Division are:

- Regression analysis
- Design and analysis of experiments
- Multivariate analysis
- Analysis of remotely sensed data
- Geostatistics
- Signal analysis

The Canberra regional group of the Division has extensive and continuing collaboration with scientists from the CSIRO Institute of Biological Resources. The appointee will be involved in strengthening and widening these contracts, through the application of statistical methodology to biological and ecological research, and the development where appropriate of new techniques.

Applicants should have a proven record of research and collaboration in relevant areas of statistics. Expertise in the sampling of biological and ecological material, and the analysis of data thus collected, are particularly relevant. Familiarity with the role and practical application of modelling in these disciplines is also desirable.

Applicants should have a PhD degree or equivalent qualifications in a relevant branch of statistics, together with appropriate research and consulting experience.

The position is available for an indefinite period and Australian Government superannuation benefits are available.

APPLICATIONS: Stating full personal and professional details, the names of at least two referees and quoting reference No A3541, should be directed to:

The Chief
CSIRO Division of Mathematics and Statistics
GPO Box 1965
CANBERRA ACT 2601
AUSTRALIA

By June 29, 1984.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W1020)A

United Medical & Dental Schools of Guy's & St. Thomas's Hospitals
NEW BLOOD LECTURESHIP
Department of Chemical Pathology

A molecular biologist with research experience is required in the Department of Chemical Pathology based at Guy's. The post offers the opportunity to work in association with a team involved in the development of malarial vaccines using recombinant DNA Technology. Applicants should submit an outline of research proposals relating to the investigation of human pathology.

Applicants wishing to visit the Department should contact Professor S Cohen (01-407 7600, ext 3365).

Appointment to this post will be for an initial period of three years. Applicants should not exceed 35 years of age and must not currently hold a permanent UK university appointment.

Salary in range £8,080 – £14,125 plus £1,186 London Allowance and Superannuation.

Application forms are obtainable from and should be returned to the Dean, Guy's Hospital Medical School, London Bridge, SE1 9RT not later than 21st June 1984.

(2172)A

INSTITUTE OF DERMATOLOGY (University of London)
Post-Doctoral Assistant

required for one year, to carry out research into analytical methods for peptide and phospholipid mediators of inflammation in human skin. An interest in modern instrumental techniques (HPLC, GCMS, FAB-MS) would be an advantage.

Salary on the Research Assistant Scale 1A.

Applications with the names of two referees to Dr A.I. Mallet, Institute of Dermatology, Homerton Grove, London E9 6BX, from whom further information can be obtained (Tel: 01-985 7061 ext 33).

(2191)A

RP FOUNDATION FIGHTING BLINDNESS

EXECUTIVE DIRECTOR

Research Foundation to prevent blindness seeks National Executive Director at headquarters in Baltimore.

Requires strong basic science background and minimum of five years administrative experience. Fund raising experience important. Unusual opportunity for the right person.

Send résumé in confidence to Marcia Snyder, Acting Executive Director, RP Foundation Fighting Blindness, 8331 Mindale Circle, Baltimore, MD 21207. (NW560)A

UNIVERSITY OF DÜSSELDORF

ASSOCIATE PROFESSORSHIP (C3) IN MICROBIOLOGY

Applicants are invited for the above position in the Institute of Microbiology, University of Düsseldorf. Candidates should be working in the field of molecular genetics of yeast or filamentous fungi to enable a close cooperation with existing research programs in the Institute. Additional funds and positions are available.

Further information can be obtained from Prof Dr C P Hollenberg, Tel 0211-3114720.

Applications, including curriculum vitae, a summary of research results and the names and addresses of three referees, should be sent to:

The Dekan der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Düsseldorf, Universitätsstraße 1, D-4000 Düsseldorf 1 (FRG).

Closing date 12 July 1984.

(W1022)A

THE UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY

LECTURER

Applications are invited for the above post available from 1 October 1984.

Salary on the academic scale for Lecturers (£7,190—£14,125) (under review) according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University of Leeds LS2 9JT, quoting reference no 83/56D. Closing date for applications 21 June 1984.

The person appointed will participate in teaching biochemistry to science and medical students and will be expected to undertake research and should be appropriately qualified. (2162)A

Gene Manipulation

Team Leader

A vacancy has arisen in a large research-based pharmaceutical company situated in the South of England for a person to lead a team of scientists involved in the application of the latest gene manipulation technology to bring about the overproduction of medically important secondary metabolites from micro-organisms. The work of this group is supported by modern, well-equipped fermentation and scale-up facilities.

To qualify for this important post the successful candidate will be an accomplished scientist with a PhD and subsequent experience in the field of genetic engineering, who should also be conversant with the methodology and potential for yield enhancement of multi-genic pathways. This challenging position is ideal for a person who feels equipped to direct this work towards a successful commercial application.

In addition to a competitive salary, benefits include a non-contributory pension scheme, free life assurance and excellent sports and social facilities. Relocation expenses will be provided where appropriate.

Please write with full personal, career and salary details to the address below, quoting **ref: S3116/N** on the envelope. Your application will be forwarded directly to the client, unless marked for the attention of our Security Manager with a note of companies to which it should not be sent.

PA Advertising

Hyde Park House, 60a Knightsbridge, London SW1X 7LE.
Tel: 01-235 6060 Telex: 27874

(2228)A

THE UNIVERSITY OF LEEDS

DEPARTMENT OF PLANT SCIENCES

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post for work on a project supported by ICI to study the growth and development of winter barley. The appointment will be made for a fixed period of up to four years, beginning as soon as possible.

Applicants should have, or shortly be expected to receive, a PhD in agricultural science or crop physiology. Experience in field studies of growth and development in cereals is desirable.

Initial salary up to £9,425 a year (under review) on the IA Scale for Research and Analogous Staff (£7,190-£11,615) (under review) according to age, qualifications and experience.

Informal enquiries may be made to Professor Elston (Tel (0532) 431751 ext 377).

Application forms and further particulars may be obtained from the Registrar, The University of Leeds LS2 9JT, quoting reference no 53/23D. Closing date for applications 25 June 1984. (2161)A

MOLECULAR BIOLOGIST - CANCER RESEARCH

The Upjohn Company, recognized as a world leader in pharmaceutical research, is seeking a Scientist with molecular biology training and experience to join its Cancer/Virus Research Department. Responsibilities will include the isolation, characterization, and utilization of cancer-related genes as part of the discovery process for new anticancer agents. Significant collaboration with departmental cell biologists in related departments is expected.

Candidates must have a Ph.D. (or equivalent degree) in biochemistry, microbiology, or related field with emphasis on the study of the molecular biology of cancer-related genes. Post-doctoral experience is desirable. Strong oral and written communication skills are required.

Upjohn offers a stimulating research environment, career potential, and peer recognition for scientific accomplishments. Publication of results and full interaction with the scientific community is expected. **For confidential consideration, send a current c.v., list of publications, names and addresses of references, and graduate transcripts (if appropriate) to: Gerald L. Potratz, Department 16278-G, The Upjohn Company, 7000 Portage Road, Kalamazoo, MI 49001.**

(NW558)A

Upjohn

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British Museum (Natural History)

Plant Taxonomist

The British Museum (Natural History) is primarily an institution for taxonomic research and has world-wide collections of animals, insects, plants and minerals. There are 5 scientific departments which are supported by a comprehensive library and full technical services including electron microscope and computer facilities.

This opportunity is in the Department of Botany which has one of the largest collections of botanical specimens in the world, providing excellent opportunities for research on the relationships, biogeography and evolution of plants. The successful candidate will continue the project for a complete typification catalogue of Linnaean plant species, and bring it to a successful conclusion. Work will also include providing an on-going Departmental service for enquiries relating to types from older collections and contributing to Flora Mesoamericana, the main Departmental flowering plant research project.

Candidates must have a 1st of 2nd class honours degree in botany or a related subject and at least 3 years relevant postgraduate experience in plant taxonomy. A knowledge of the older literature and collections would be especially advantageous.

Appointment as Higher Scientific Officer £8,395-£10,810 (including £1250 Inner London Weighting) with starting salary according to qualifications and experience.



For further details and an application form (to be returned by 22 June 1984) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours).

Please quote ref SB/78/DK.

The Civil Service is an equal opportunity employer

(2211)A

UNIVERSITY OF ABERDEEN
DEPARTMENT OF MICROBIOLOGY

TEMPORARY LECTURESHIP

The Department of Microbiology invites applications for a Temporary Lectureship for one year from October 1984. The post arises from the award of the Nuffield Research Fellowship to Dr I R Booth, and the successful applicant will be expected to assist with teaching in microbial physiology, genetics and molecular biology. There will be the opportunity also to carry through a research programme.

Salary within the range £7,190 to £8,530 per annum, on lecturers salary scale (under review).

Further particulars and application forms from the Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 15 June, 1984. (2139)A

UNIVERSITY OF LEICESTER
Leicester Biocentre

POSTDOCTORAL RESEARCH ASSOCIATE
Yeast Molecular Biology

Applications are invited for a postdoctoral research position, funded by a major UK Food and Drink company, to undertake research aimed at the improvement of existing yeast strains for commercial applications.

The project will involve the use of genetical, biochemical and recombinant DNA methods and Applicants should hold a PhD in a relevant subject. The contract is for a period of 3 years and appointment will be made within the Grade 1A salary scale (ie £7,190 — £11,615).

Applications, with full curriculum vitae should be sent to the Secretary, Leicester Biocentre, University of Leicester, Medical Sciences Building, Leicester LE1 7RH. (2105)A

DEPARTMENT OF BIOLOGY

Postgraduate Research Training

Three opportunities for research training, leading to MPhil/PhD (CNAA), will exist in the department from 1 September 1984. The following project areas are available:

Photoperiodism and Reproduction; Feedback control mechanisms on ion absorption and transport in higher plants; Allosteric models and haemoglobin; Production and characterisation of monoclonal antibodies for use in a diagnostic kit to detect demyelination; Biochemical mechanisms of resistance to aminoglycosides in clinically isolated bacteria; Laboratory selection for pesticide resistance in predatory mites; Glycoprotein metabolism in nerve terminals; *In vitro* construction of Tn5-derived transposons carrying novel selective markers and transactivated oriT regions; Physiological and biochemical indicators of alcohol consumption in support of clinical therapies; Numerical aspects of species diversity in insects.

Further details of the projects and of research in the Department available from the Postgraduate Tutor. Two of the posts are Researcher A appointments (£4,947 pa under review) associated with some teaching duties in physiology and biochemistry respectively.

Applicants should possess a good honours degree in an appropriate subject. They should provide a curriculum vitae including full details of any experience relevant to the project they nominate, and the names of two academic referees. Applications should be sent as soon as possible to:

The Postgraduate Tutor, Department of Biology, Oxford Polytechnic.

DEPARTMENT OF GEOLOGY AND PHYSICAL SCIENCES

Temporary Lecturer II/ Senior Lecturer in Geology Ref: GPS/ 31

Applications are invited for a one-year, full time temporary post in the Department from suitably qualified candidates. The post will be available from 1 September 1984 to 31 August 1985.

The successful candidate will in part, be resonsible for the teaching of Petrology and Mineralogy as part of the Polytechnic's modular degree course. Petrology and Mineralogy are taught within the named degree in Earth Sciences and the Geology single field honours degree.

Teaching responsibilities will be in at least two of the following areas: Metamorphic Petrology; Igneous Petrology with special reference to Plutonic Rocks; Introductory Mineralogy and Petrology. In addition the successful candidate will be required to supervise undergraduate field trips, may be required to teach geological map work and will be encouraged to participate in research.

Salary Scale
LII £7,215 — £11,568

Salary Scale
SL £10,683 — £13,443
under review)

Further details and application forms are available from the Staffing Department, ext 363.

The closing date for these posts is 15 June 1984. (2193)A

THE UNIVERSITY OF IOWA
DEPARTMENT OF CHEMISTRY

POSTDOCTOROAL POSITIONS (2) — ENZYME MECHANISMS

Two postdoctoral positions are available beginning July 1, 1984, for persons with research experience and interests in physical biochemistry and/or enzyme biochemistry. One position is to study the mechanisms of the lipolytic enzymes cholesteryl esterase and lipoprotein lipase. The second position is to study the mechanism of acetylcholinesterase (see J Amer Chem Soc (1984) 106, 1883-1884). Both positions involve the design and synthesis of substrates for the studied enzymes and the states by a range of reaction kinetics probes, including: solvent and substrate isotope effects, temperature and pH effects, effects of organic cosolvents, nucleophilic trapping of intermediates. The lipolytic enzume and study of enzyme activation by artificial membranes. Experience in enzyme kinetics and/or purification is necessary. Some experience in synthetic organic chemistry is desirable but is not required. Please send résumé and arrange for two letters of recommendation to be sent to:

Dr Dan Quinn, Department of Chemistry, The University of Iowa, Iowa City, Iowa 52242. Phone: 319-353-6398

The University of Iowa is an Affirmative Action/Equal Opportunity Employer. (NW561)A

ASSISTANT PROFESSOR of Immunology and Infectious Diseases. (Parasitology). The Johns Hopkins University invites applications for this position from candidates who have strong interest in the parasitic diseases. Curriculum vitae, description of research goals and interests, and the names, addresses, and telephone numbers of three referees should be submitted to: The Chairman, Department of Immunology and Infectious Diseases, The Johns Hopkins University School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, Md 21205. The Johns Hopkins University is an Equal Opportunity Employer. (NW566)A

Please mention

nature

when replying to

these advertisements

ADJUNCT ASSISTANT/Associate Professor: PhD with demonstrated productivity in molecular biology mutagenesis DNA repair, or replication experts given slight preference). Full-time research, optional teaching. Postdoctoral Associates: to study molecular basis of mutagenesis; opportunity to learn state-of-the-art DNA technology. The Department of Microbiology, with ten vigorous, young groups devoted to molecular genetics, offers an outstanding academic environment. The location permits easy access (3 to 10 miles) to the attractive suburbs and to the many cultural and recreational amenities of New York City (10 miles). Attractive terms. Apply to: Dr Z Humayun, Department of Microbiology F607, New Jersey Medical School, 100 Bergen Street, Newark, NJ 07103. (NW553)A

university college of swansea

Research Demonstrator

Applications are invited for the vacancy of **Research Demonstrator** in the **Department of Geology**. Applicants should either be in possession of a PhD or have such a degree near to completion. Special interest in mineralogy, petrology and/or structural geology would be an advantage.

The appointment, which will be for one year in the first instance from September 14, 1984, will be on a scale up to £8,530 per annum together with USS/USDPS benefits.

Further particulars and application forms (2 copies) should be obtained from the Personnel Office, University College of Swansea, Singleton Park, Swansea SA2 8PP, to which office they should be returned by *Friday, June 22, 1984.* (2223)A

Cedar Crest College

MOLECULAR BIOLOGIST

Tenure-track position, assistant professor, starting September 1984. PhD degree with teaching and research experience in recombinant DNA and hybridoma technology. Prefer applicants with related postdoctoral experience. Successful candidate will direct undergraduate program in biotechnology, teach courses related to the program, and supervise undergraduate research. Salary competitive.

Applications close July 1, 1984. Submit résumé, three letters of reference and official transcripts to Dr Marion Kayhart, Chairperson, Department of Biology, Cedar Crest College, 30th and Walnut, Allentown, PA 18104. (NW554)A

COMMONWEALTH AGRICULTURAL BUREAUX

The leading world agricultural information service for scientists and technologists

SCIENTIFIC INFORMATION OFFICERS

There are two vacancies at the Commonwealth Bureau of Patures and Field Crops, Hurley, Nr Maidenhead.

Duties include scanning and abstracting the world literature on annual field crops, grasslands, herbage plants, weeds and weed control for publication in CAB database.

Degree in agriculture or biological sciences essential. Reading knowledge of at least one language other than English desirable. Salary in scale £5,682-£9,561 per annum. Starting salary in accordance with qualifications and experience.

Closing date for applications: 30 June, 1984.

For application forms and further details, write or telephone to: Commonwealth Bureau of Pastures and Field Crops, Hurley, Maidenhead, Berks SL6 5LR. Tel. Littlewick Green 3457.

50 YEARS' SERVICE · CAB · TO AGRICULTURE

(2201)A

SCRIPPS CLINIC AND RESEARCH FOUNDATION

TWO POSTDOCTORAL RESEARCH POSITIONS

are available for 2 years in an established Hepatitis B virus research laboratory. A molecular biologist position requiring PhD or MD and experience in basic recombinant DNA techniques, DNA mediated gene transfer, and cell culture will investigate the state and expression of the Hepatitis B viral genome in human and animal systems. An immunologist position requiring PhD and experience in T cell cloning techniques will investigate cellular aspects of the immune response to HBV antigens.

Please submit cv, references and a statement of research interests to Dr F V Chisari, C-202, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037. (NW552)A

RESEARCH PHYSICIAN — Medical research in autoimmune diseases and ocular microbiology; review and retrieve data in medical fields' prepare research proposals; write and edit scientific articles; medical Doctor's degree; one year medical research experience required; passed FLEX exam; Job located in San Francisco, $22,000 per year; Send résumé and this ad to Job No 9081, PO Box 865, Sacramento, CA 95804, not later than June 15th. (NW572)A

AGRICULTURAL AND FOOD RESEARCH COUNCIL

FOOD RESEARCH INSTITUTE (Chemistry and Biochemistry Division)

PHYSICIST/BIOPHYSICIST BIOCHEMIST/CHEMIST

As a result of additional funding by the Ministry of Agriculture, Fisheries and Food, the following posts are available in the Chemistry and Biochemistry Division. The posts are aimed at expanding the commitment in research into Biopolymers already in the Division. There is substantial existing expertise in the isolation and characterisation of proteins and polysaccharides. There is also a major emphasis on the use of modern physical methods to study molecular behaviour. The methods available on site include: light-scattering, X-ray diffraction, low-angle X-ray scattering, electric birefringence, flow birefringence, multinuclear NMR spectroscopy, high-power NMR spectroscopy (including MAS facility), differential scanning calorimetry, dilatometry and electron microscopy. Links with specialist University departments are established to extend further the range of available techniques.

The posts are concentrated in two areas of research.

A. THE FUNCTIONAL PROPERTIES OF CEREAL PROTEINS (Posts 84/NP/15, 84/NP/16 and 84/NP/17):

The aim of the research is to determine the structural factors that control the performance of cereal proteins, especially gluten, in food, e.g., in baked products. The programme will involve isolation and characterisation of cereal proteins, determination — by physical and spectroscopic methods — of their structures, including structural changes as a result of denaturation, and the study of physicochemical and rheological behaviour of protein solutions and gels related to changes of molecular structure.

B. THE PROPERTIES OF STARCHES (Posts 84/NP/4, 84/NP/5 and 84/NP/6):

The aim is to identify the molecular processes that determine the stability of starch and startch-protein gels. The programme will involve the study of (i) the solution behaviour of well-characterised starch polysaccharides, using light-scattering and viscometry; ((ii) the molecular association in concentrated systems, using X-ray diffraction, dilatometry and differential scanning calorimetry; and (iii) related studies in changes in mechanical behaviour.

Qualifications: Details of the posts are as follows:

PROTEIN BIOCHEMIST, Higher Scientific Officer (84/NP/15)
A first or upper second-class honours degree in Biochemistry and at least two years' postgraduate experience, preferably a Ph.D., in Protein Biochemistry or Enzymology. Expertise in the characterisation of proteins is essential and ability to interact with biophysicists in a multidisciplinary team, desirable.

BIOCHEMIST, Scientific Officer (84/NP/16)
A degree in Chemistry or Biochemistry. Aptitude for careful manipulation of sensitive biopolymers is essential.

PHYSICAL CHEMIST/SPECTROSCOPIST, Scientific Officer (84/NP/17)
A degree in Physical Chemistry. Experience with the use of physical or spectroscopic methods in biological systems is desirable.

BIOPHYSICIST/PHYSICAL BIOCHEMIST, Higher Scientific Officer (84/NP/4)
A first of upper second-class honours degree in Physics, Physical Chemistry or a related subject and at least two years' postgraduate experience, preferably a Ph.D., in the molecular behaviour of proteins or polysaccharides. Experience with using physical techniques to study complex biopolymeric systems is essential.

PHYSICAL CHEMIST/PHYSICIST, Scientific Officer (84/NP/5)
A degree in Physical Chemistry or Physics. Interest in the application of physical methods to the study of biological systems is essential.

PHYSICIST/PHYSICAL CHEMIST, Scientific Officer (84/NP/6)
A degree in Physics of Physical Chemistry. Experience with the study of the bulk rheology of systems is desirable.

Salary scales: Higher Scientific Officer £7,149-£9,561, Scientific Officer £5,682-£7,765 (under review).

Non-contributory superannuation scheme.

The Agricultural and Food Research Council is an Equal Opportunities Employer.

Further information and application forms from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA, quoting the appropriate reference number. Closing date: 15th June, 1984. (2209)A

ROYAL DUTCH/SHELL GROUP OF COMPANIES

Production/ Development Geologists

International career opportunities

Production Geologists are responsible for all geological matters relating to the appraisal and development of oil and gas discoveries and work in close cooperation with other branches of petroleum engineering. They take a leading part in field appraisal and the resultant pre-development plans, siting of development wells and construction of detailed geological models for use by the Reservoir Engineers.

Minimum requirements are an upper second class degree in geology, and at least four years relevant experience. An additional higher degree will be regarded as evidence that candidates are fully equipped to take advantage of longer term career prospects. Applicants must be familiar with the principles of petroleum geology, and should have an extensive knowledge of well log correlation, subsurface mapping techniques, seismic interpretation and geological analysis of clastic and/or carbonate reservoirs. A basic knowledge of general petroleum engineering and data processing would be an advantage.

We offer a career with excellent prospects for development and promotion. Starting salaries will be commensurate with background and experience. Applicants must be prepared to work overseas for a large part of their career. Benefits will include attractive family accommodation, primary schooling or assistance with boarding school fees, free annual leave passages, good recreation and medical facilities, and an excellent pension scheme.

Please write for an application form to:

(2219)A

Shell International Petroleum Company Limited, Recruitment Division (N1), PNEL/22, Shell Centre, London SE1 7NA.

THE UNIVERSITY OF GLASGOW

DEPARTMENT OF BIOCHEMISTRY

POSTGRADUATE RESEARCH ASSISTANT

A position is available, under a grant from the Muscular Dystrophy Group of Great Britain, for a research assistant to pursue recombinant DNA studies in relation to Duchenne Muscular Dystrophy commencing 1st October, 1984.

Applicants should have, or expect to gain, a first or upper second class honours degree in Molecular Biology, Genetics, Biochemistry or other relevant biological science; and the appointee will register for a PhD. The work involves construction of genomic DNA libraries and identification of X-chromosome specific cDNA clones.

Letters of application, including curriculum vitae and the names of two referees, should be addressed to: Dr D P Leader, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ. (2168)A

FACULTY POSITION

MOLECULAR GENETICIST

The Division of Clinical Immunology and Rheumatology is inviting applications for a tenure-track position at the assistant/associate professor level. A strong commitment to research excellence will be the main criterion of selection. Training in molecular biology is required and research interests in the areas of immunology or mechanisms of inflammation preferred. Candidates must have demonstrated research ability and will be expected to establish a strong independent research program. preference will be given to candidates holding an MD degree. opportunities for participating in graduate programs of Basic Science Departments are available. Curriculum vitae, statement of research goals and interests and names of three referees should be sent by August 1, 1984, to: John E Volanakis, MD, Molecular Geneticist Search Committee, Division of Clinical Immunology and Rheumatology, University of Alabama in Birmingham, Birmingham, Alabama 35292. An Equal Opportunity/Affirmative Action Employer. (NW568)A

UNIVERSITY OF STRATHCLYDE

"New Blood" Lectureship
In Food Science Division
Department of Bio-science and Bio-technology

Applications are invited from candidates under 35 years of age for a lectureship in food science. Preference will be given to candidates with post-doctoral research experience in food related topics. Experience in one or more of the following areas will be an advantage:-

Biopolymers, rheology, food preservation, food microbiology, food processing and control systems.

Salary will be on a lecturer scale (£7,190 – £14,125 p.a.) USS Benefit.

Further particulars and application forms (quote ref 30/84) are available from Staff Office, McCance Building, 16 Richmond Street, Glasgow G1 1XQ.

Closing date for applications: 29 June 1984. (2227)A

RESEARCH ASSOCIATE POSITION AVAILABLE

Research program is in the area of molecular basis of calcium action, especially the mechanism of action of the calmodulin family of proteins. Background in Molecular Biology or Biochemistry preferred, but motivation and ability to interact with a multidisciplinary group more important. Send curriculum vitae and names of two references to D Martin Watterson, Department of Pharmacology and Howard Hughes Medical Institute, 723 Light Hall, Vanderbilt University, Nashville, TN 37232. (NW573)A

Neurobiologist

Postdoctoral position available to study chemosensory transduction in vertebrate taste cells. Research involves intracellular recording and dye-filling, patch-clamping, structure/function correlations with electron microscopist. Experience in electrophysiology, particularly intracellular microelectrodes desirable.

Contact Dr S. Roper, Rocky Mt. Taste and Smell Center, B-111, University of Colorado Medical School, 4200 E. 9th Ave., Denver, CO 80262. Affirmative action/Equal opportunity employer. (NW567)A

CENTRE FOR REPRODUCTIVE BIOLOGY,
UNIVERSITY OF EDINBURGH

GYNAECOLOGIST

Applications are invited from gynaecologists for a clinical post established by the Medical Research Council to promote the research and development of new approaches to contraception and menstrual induction, and the treatment of reproductive disorders. A Honorary Clinical Contract will be arranged with the local Health Authority for the successful candidate. The terms of this contract are negotiable, but will be designed to provide access to patients, both for research and for the development of the candidate's clinical career. Outstanding opportunities exist for clinical research in the Simpson Memorial Maternity Pavilion, adjacent to the Centre for Reproductive Biology, and for collaboration with the Scientific Staff of the Centre. Applicants should have their MRCOG and have undertaken some research.

The appointment will be made on the MRC Clinical Scales, at the equivalent of Senior Registrar or Consultant Grade.

Applications, including a full curriculum vitae, list of publications and the names of three referees should be sent by 25th June to Dr D W Lincoln, Director, MRC Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW (telephone number 031 229 2575).

(2215)A

Tropical Development and
Research Institute, London

Food Scientist/ Microbiologist

... to join a team concerned with: the application in developing countries of food quality control/quality assurance; low cost methods of food preservation; and utilisation of agricultural products by fermentation. The work involves some overseas travel.

Candidates must have a 1st or 2nd class honours degree in food science, microbiology or biochemistry and at least 2 years postgraduate experience of food microbiology/fermentation.

Appointment as Higher Scientific Officer £8395-£10,810 (including £1250 Inner London Weighting) with starting salary according to qualifications and experience. An additional allowance is payable for working overseas.

For further details and an application form (to be returned by 22 June 1984) write to Mr D G Cornish, Tropical Development and Research Institute, 127 Clerkenwell Road, London EC1R 5DB.

Please quote ref: SA/11/JE.

The Civil Service is an equal opportunity employer

Scientific Civil Service

(2210)A

Clinical Research

Antibiotics
Excellent salary + car

Roussel Laboratories is part of the world's largest and most innovative ethical pharmaceutical company.

The medical department, based at our Wembley Park head office (moving later this year to Denham, Bucks), is seeking a Senior Clinical Research Scientist to join its team of medical advisers, scientists and statisticians. This is a unique opportunity for someone to develop their skills working on post marketed antibiotics and projects, and to build up contacts with opinion leaders in the medical field by organising clinical trials and symposia.

Probably in your 30s with a BSc, MSc or PhD, preferably in microbiology, you will ideally have at least five years' experience in a similar role with a pharmaceutical company, working on antibiotics. You must have a strong interest in post marketed products and possess the necessary resourcefulness, drive, and contacts in the industry and the medical field to enable you to handle projects alone.

We provide a first-class range of benefits including free family BUPA after a year's service, free restaurant, free life and personal accident insurance, contributory pension, and generous relocation assistance where appropriate.

Please send a detailed cv, including current salary, or ring or write for an application form to Mrs Elizabeth Bedford, Roussel Laboratories Limited, Roussel House, Wembley Park, Middlesex HA9 0NF. Telephone: 01-903 1454.

ROUSSEL

(2199)A

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

(University of London)
London W1P 6DB

Applications are invited for the post of

LECTURER IN ANATOMY

Applicants should have experience in gross anatomy teaching and the successful candidate will be encouraged to help in the development of the gross anatomy teaching programme. Candidates should also be committed to research and will be encouraged to develop their own research programme. Current research interests in the Department range over Developmental Biology, Hominid Palaeontology, Comparative Anatomy, Cell Membranes and the Ultrastructure of Peripheral Nerves. Applications are particularly invited from candidates whose research interests are allied to Hominid Palaeontology and Evolutionary Biology.

Salary according to age, qualifications and experience within the range £8,376 — £15,312 inclusive of London Allowance. Further details may be obtained from Professor Bernard Wood (Tel 01-636 8333 ext 7179).

Applications including cv and names and addresses of three referees to Professor B A Wood, Department of Anatomy and Biology at the above address as soon as possible.

(2184)A

UNIVERSITY OF DUNDEE

CHAIR OF BIOCHEMISTRY

Applications are invited for the Chair of Biochemistry which will fall vacant on 1 October 1984 as a result of the resignation of Professor P B Garland to take up a senior research post in industry.

Further particulars are available from The Secretary, The University, Dundee DD1 4HN with whom applications (10 copies, or if posted overseas one copy) containing full career details, a summary of research interests, and the names of three referees should be lodged as soon as possible. Please quote reference EST/18/84. It is intended that short-listed candidates should be interviewed in Dundee before the end of September, 1984 (J). (2189)A

CHAIRPERSON

DEPARTMENT OF ANATOMY

Applications and nominations are invited for the position of Professor and Chairperson, Department of Anatomy, University of South Carolina School of medicine, Columbia, South Carolina. Candidates should have a record of scholarly activity, including excellence in teaching, preferably in gross anatomy, active research interest and administrative and leadership skills. Applications and nominations will be accepted until June 30, 1984. Please submit to: O'Neill Barrett, Jr., MD, Chairman, Anatomy Search Committee, USC School of Medicine, c/o Richard Memorial Hospital, ACC No 2, Columbia, SC 29203. An Equal Opportunity/Affirmative Action Employer. (NW571)A

Post Doctoral, Research Scientist

Department of Haematology and Oncology

Royal Children's Hospital Melbourne, Australia

An opportunity exists to undertake a leading research role in the fields of childhood leukaemia and childhood solid tumours for a scientist with experience in this field. The research laboratory, part of the department of Clinical Haematology and Oncology undertakes the care of more than 400 children with a variety of leukaemias and solid tumours and is the paediatric centre for bone marrow transplantation and autologous bone marrow rescue. Development and research are well established in the department and a list of research activities and publications will be forwarded on request. The Royal Children's Hospital is a major Paediatric centre in Australia and very good scientific support facilities are available in virtually all disciplines including immunology and recombinant DNA technology. Joint research projects with the Walter and Eliza Hall Institute and the Ludwig Cancer Research Institute are well established. Technical help will be available and there is scope for training of doctoral fellows.

The successful candidate will be funded at the appropriate N.H. and M.R.C. scale for seniority and experience (range $A20,164.00-$A30,737.00). Funds are available for a three year appointment, however transport and removal expenses cannot be provided.

Written applications describing the candidates area of interest, expertise, the proposed research programme for the three years, a curriculum vitae and the names and addresses of at least two referees, should be addressed to Dr. H. Ekert, Department of Haematology and Oncology.

Applications close Friday, 20th July, 1984.

Dr. Barry R. Catchlove,
Chief Executive.

(2177)A

0412

Royal Children's Hospital

Flemington Road, Parkville 3052, Victoria, Australia.

CSIRO
AUSTRALIA
RESEARCH FELLOW
$A25,342-$A31,270
DIVISION OF SOILS
CANBERRA ACT

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Soils is a member of the Institute of Biological Resources.

GENERAL: The CSIRO Division of Soils studies the physics, chemistry and biology of soils and other porous media, together with the integrative disciplines of pedology and geomorphology. It also seeks to establish principles for the application of soil science to agriculture, forestry, hydrology, engineering and conservation. The Division has laboratories in Adelaide, Brisbane, Canberra and Townsville.

Micromorphological studies are used in the interpretation of soil genesis and in the explanation of many soil properties and forms of behaviour. Facilities for micromorphological studies at the Canberra Laboratories include comprehensive thin-sectioning equipment, a range of binocular and polarizing microscopes, computerised quantitative image-analysis systems, a rainfall tower for the simulation of field conditions and a wide range of anciliary facilities in the sub-disciplines of soil physics and chemistry. Access to a scanning electron microscope and electron microprobe is also available within the Division.

DUTIES: The appointee will undertake research on relationships between micromorphology and the physical and mechanical properties of soils. In particular, the studies will focus on the relationship between soil structure and hydraulic conductivity, compaction and shear in clay soils, using dyes, direct shear-box tests and quantitative image-analysis. There will also be opportunities to assist with general micromorphological studies of soil A horizon properties and soil genesis.

QUALIFICATIONS: A PhD degree or equivalent qualifications in soil science, physical geography, or geology with basic skills in microscopy and preferably some experience in soil physics and pedology.

LOCATION: The appointee will be based in Canberra, ACT.

TENURE: A term of 5 years. Australian Government superannuation benefits available.

APPLICATIONS: Stating full personal and professional details, the names of at least two referees and quoting reference No A3269, should be directed to:

The Chief
CSIRO Division of Soils
GPO Box 639
CANBERRA ACT 2601
AUSTRALIA

By June 29, 1984.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W1019)A

The International Livestock Centre for Africa (ILCA) has a vacancy for an

Agricultural Economist

in ILCA's Humid Zone Programme based in Ibadan, Nigeria, where the research focuses on small ruminants. The appointee will be a member of a multi-disciplinary team and will carry out field surveys and analyse and evaluate existing production systems and proposed changes in these.

The successful candidate will have a PhD in agricultural economics or an MSc plus several years experience working on smallholder farming systems in developing countries.

For a person recruited internationally the appointment will be initially for two years, with an attractive tax-free salary payable in US$ outside Nigeria. In addition to salary various allowances are payable.

Applicants should send a curriculum vitae and the names of three referees by June 30, 1984 to:

The Director General
ILCA, P.O. Box 5689
Addis Ababa, Ethiopia

Addis Ababa, 26 April 1984. (W1018)A

Population Biologist

The Sea Mammal Research Unit is seeking a Biologist or Mathematician to join a small team developing and refining techniques for estimating the size and status of seal and whale populations. The research will involve both mathematical and statistical modelling and fieldwork, the latter often under unpleasant conditions. Experience of the application of a variety of mathematical techniques to biological problems, work with large mammals in the field, and handling of small boats would be an advantage.

Appointment will be at the Scientific Officer grade (£5,682 — £7,765 pa) or Higher Scientific Officer grade (£7,149 — £9,561 pa), depending on qualifications and experience.

For further details and an application form write to the Sea Mammal Research Unit, c/o British Antarctic Survey, High Cross, Madingley Road, Cambridge CB3 0ET quoting post no SM/10.

Closing date for applications: 21st June 1984. (2220)A

NATURAL ENVIRONMENT RESEARCH COUNCIL

UNIVERSITÄT ULM

An der Fakultät für Theoretische Medizin ist die

C4 Professur für Virologie

(Nachfolge Prof Dr A.K. Kleinschmidt) zu besetzen. Mit der Professur ist die Leitung der Abteilung Virologie (Mikrobiologie I) verbunden. Der Stelleninhaber hat das Fach Virologie in Forschung und Lehre sowie in der mittelbaren Krankenversorgung zu vertreten.

Wissenschaftlich sollte er in molekularbiologischer Forschung ausgewiesen sein; Beteiligung an einem zellbiologisch orientierten SFB sowie an der Lehre in Biologie für Fortgeschrittene wird erwartet.

Bewerber werden gebeten, die üblichen Unterlagen bis zum 2. Juli 1984 an den Dekan der Fakultät für Theoretische Medizin der Universität Ulm, Postfach 4066, D-7900 Ulm (Donau), zu senden. (W1017)A

M.R.C. CLINICAL RESEARCH CENTRE
(NORTHWICK PARK HOSPITAL)
WATFORD ROAD, HARROW
MIDDX HA1 3UJ

POSTDOCTORAL FELLOW IN MOLECULAR IMMUNOLOGY

Applications are invited from science graduates for a 3-year postdoctoral position in the transplantation biology section to work with Dr A. Mellor on a project to clone and characterise human and mouse genes which encode minor histocompatibility antigens.

Candidates should have previous experience with recombinant DNA techniques. A knowledge of immunological techniques would be helpful but is not essential.

Salary scales are related to those for University Non-Clinical Academic Staff and salary on appointment will depend on age and experience. For an application form write to Miss J. Cottam, Personnel Section quoting reference 129/ 1/ 4517. Closing date for applications, 22nd June 1984. (2181)A

THE UNIVERSITY
OF SHEFFIELD
DEPARTMENT OF
PHYSICS

1 RESEARCH ASSOCIATE
and
2 PHYSICIST
PROGRAMMER IN HIGHER
ENERGY PHYSICS

Applications are invited from men and women for the above SERC funded posts, tenable for two years from 1 October 1984, with the possibility of a third year.

Post 1 concerns a photoproduction experiment using a tagged photon beam and Omega detector at CERN.

Post 2 involves work in two areas: the on-line system for the ALEPH experiment associated with the LEP project at CERN and off-line analysis using the University's Workstation.

A good honours degree in physics is required together with postgraduate experience in the experimental techniques of particle physics. Initial salary £7,190 — £9,425 a year on range IA according to age, qualifications and experience.

Applications, including curriculum vitae and the names of two referees, should be sent to Professor W Galbraith/Dr F Combley, Department of Physics, The University, Sheffield S10 2TN, by 15 June 1984. Quote ref: R95/. (2224)A

RECENT PhD BIOCHEMISTRY — Protein Chemistry/Enzymology. Experienced with many laboratory techniques such as immunochemistry, isoelectric focusing, etc., and instruments such as CD, NMR, fluorometry, etc. Seeks position as postdoctoral/research associate/teaching. Available immediately. Reply to Box 42, Nature, 15 East 26th Street, New York, New York 100100, USA. (NW570)A

UNIVERSITY
OF ST ANDREWS
DEPARTMENT OF
ANATOMY & EXPERIMENTAL
PATHOLOGY

RESEARCH ASSISTANT

Applications are invited from recent graduates for a post of Research Assistant required to assist in the production of monoclonal antibodies against malignant melanoma cells. Experience in membrane biochemistry, cell culture or monoclonal antibody production would be advantageous. The appointment is funded by a grant from the NFCR and will be for one year in the first instance with the prospect of renewal.

Starting salary £6,310 per annum (under review) plus USS.

Applications with full curriculum vitae and the names of two academic referees should be sent as soon as possible to Dr Clive W Evans, Department of Anatomy and Experimental Pathology, University of St Andrews, St Andrews, Fife KY16 9TS, from whom further particulars may be obtained. (2214)A

Yeast Microbiologist

Top salary + car

Burton upon Trent, Staffordshire

Bass, Europe's largest brewing group, wish to appoint an experienced specialist who will be responsible to the Quality Control Director. He/she will provide a microbiological service to all Bass breweries and supervise activities of the Group QC laboratory.

Duties will include supervision of the Bass Yeast Quality Assurance Policy, advice to breweries in respect of yeast propagation systems and yeast husbandry. Also assessment of new/improved microbiological techniques to meet specific QC needs.

Applicants with specialised knowledge of brewing yeasts and propagation systems are required to have a minimum qualification of B.Sc. Hons. 2(1) in Microbiology or a related discipline and have at least 5 years post graduate experience in the brewing/fermentation industries. Experience and enthusiasm for microbiological trouble shooting would be advantageous.

Salary will be commensurate with age and experience. The Company operates a generous life assurance and pension scheme and provides comprehensive recreational facilities.

Detailed application including c.v. and names of two referees, should be sent as soon as possible to:-

HQ Staff Personnel Manager, Bass Public Limited Company, 137 High Street, Burton upon Trent DE14 1JZ

Bass
Public Limited Company

THE UNIVERSITY
OF LEEDS

DEPARTMENT OF
ANATOMY

LECTURER

Applications are invited for the above post available from 1 September 1984.

Qualifications in Medicine, Basic Medical Sciences or Biology and research interests in Morphometry, Human Evolution or Cell Biology are required.

Salary on the academic scale for Lecturers (£7,190 — £14,125) (under review) according to age, qualifications and experience.

Informal enquiries may be made to Professor W J Moore (0532) 431751 ext 7547.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference no 82/8/D. Closing date for applications 22 June 1984. (2188)A

UWIST
THE WELSH SCHOOL OF PHARMACY

APPLIED BIOLOGY RESEARCH ASSOCIATE (fixed term — 2 years)

supported by the National Kidney Research Fund to work on the regulation of intracellular protein degradation in kidney epithelial cell culture. Experience in tissue culture is desirable.

Salary: Within Range 1A Research and Analogous Staff £7,190 — £8,080 per annum.

Requests (quoting Ref B67) for details and application form to Staffing Office, UWIST, PO Box 68, Cardiff CF1 3XA.

Closing date: 21 June 1984.
(2151)A

ADAS

Agricultural Science Service

ANALYTICAL CHEMISTS

The Agricultural Science Service provides farmers and growers in England and Wales with technical information and advice on agricultural and horticultural matters and undertakes scientific investigational and development work. There are currently opportunities for analytical chemists at the Aberystwyth and Cardiff laboratories. You will be involved in examining and analysing feedingstuffs, plant materials, soils and other agricultural materials, and assisting in the development of techniques and apparatus.

You should have a degree or equivalent in chemistry preferably with emphasis on analytical techniques. Consideration will be given to candidates possessing degrees or equivalent in other branches of chemistry provided they possess practical experience relevant to the post.

Appointments as Scientific Officer £5680-£7765 with starting salary according to qualifications and experience.

For further details and an application form (to be returned by 22 June 1984) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote ref: SB/33/AL.

The Civil Service is an equal opportunity employer

Ministry of Agriculture, Fisheries and Food (2212)A

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)

POSTDOCTORAL RESEARCH FELLOW: ARC BONE & JOINT RESEARCH UNIT

Applications are invited for the post of Research Fellow to work on production and characterization of monoclonal autoantibodies in rheumatoid arthritis by human-human hybridisation. The project is funded for three years (one year in the first instance) by a grant from the Arthritis and Rheumatism Council.

Applicants should have experience in tissue culture and immunological techniques. Salary £7,190 — £8,080 (University of London Range IA) plus £1,186 London Weighting.

Applications with cv and names of two referees to Professor HLF Currey, ARC Bone and Joint Research Unit, The London Hospital Medical College, 25-29 Ashfield Street, London E1 2AD. Informal enquiries may be made to Dr J R Archer on 01-247 8939.

(2152)A

TARONGA ZOO, SYDNEY, AUSTRALIA

Applications are invited for the position of

AQUARIUM CURATOR

Duties:
To provide scientific management of a large, diverse aquatic collection. And to participate in the design, construction and implementation of a new aquatic complex.

Qualifications:
A degree in Zoology or related field is essential. Extensive management experience in a large aquarium or similar facility is preferred.

Conditions of Service:
Similar to those of the New South Wales Public Service.

Salary Range:
Available on application.

Apply to: Director,
TARONGA ZOO,
P.O. Box 20,
Mosman, NSW 2088,
Australia.

Applications close 30 June 1984. (W1015)A

POSTDOCTORAL POSITION available 1 July 1984 for a PhD trained in one of the following disciplines: biochemistry, microbiology, molecular biology, or biochemical pharmacology. The fellow will pursue the cloning structure, and regulation of the mammalian gene(s) encoding DNA polymerase alpha. Experience with immunologic techniques, tissue culture, and recombinant DNA technology — including cDNA cloning of low abundance mRNA — is preferred. Salary will be competitive and commensurate with experience. Send curriculum vitae and three references to: Dr Neal C Brown, Professor and Chairman, Department of Pharmacology, University of Massachusetts Medical School (UMMS), 55 Lake Avenue North, Worcester, Mass 01605. UMMS is an Affirmative Action/Equal Opportunity Employer.

(NW565)A

UNVERSITY SCHOOL OF MEDICINE
DEPARTMENT OF MEDICINE

POSTDOCTORAL FELLOWSHIPS IN IMMUNOLOGY

funded by an NIH training grant to the Interdepartmental Immunology Program at Tufts University School of Medicine are available. Appointments will be for one year with the possibility of an additional year. Research areas include immunobiology, molecular biology of the immune response, immunogenetics, immunochemistry, cancer immunology and clinical immunology with an emphasis on interdisciplinary work.

Please submit a curriculum vitae, a brief summary of research interests and names of two references to Dr Lanny J Rosenwasser, Department of Medicine, Tufts University School of Medicine, 171 Harrison Avenue, Box 71, Boston, Massachusetts 02111. (NW576)E

Nestlé

Our Central Research Department in Vevey/Switzerland is seeking a

chemist or biochemist

to work in the protein field.

Activity

- Analysis of food proteins by electrophoretic and chromotographic techniques.
- Extraction, fractionation and purification of proteins from various raw materials on laboratory and pilot plant scale.
- Study of physicochemical and functional properties of food proteins.

Qualifications required

- University degree with Ph.D.
- Experience in protein purification and characterization.

Please send your application for employment with curriculum vitae and photograph to:

Miss Myriam Germanier
Personnel Services,
Nestlé Products Technical Assistance Co. Ltd.,
1800 Vevey/Switzerland.

(W997)A

Nestlé

UNIVERSITY OF OTAGO
Dunedin, New Zealand

LECTURERS IN ZOOLOGY

Applications are invited for two Lectureships in the Department of Zoology. Persons appointed will be required to contribute to general undergraduate courses in biology and zoology and to teach and conduct research in specialist aspects of the subject. One post is in terrestrial vertebrate ecology and population biology, the other in experimental zoology and comparative physiology of invertebrates or lower chordates.

Salary: Lecturer: NZ$21,660-$25,684 per annum, plus NZ$8.00 per week cost-of-living allowance.

Further information, including method of application, is available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, University of Otago, PO Box 56, Dunedin, New Zealand.

Applications close on 31 July 1984. (2204)A

UNIVERSITY OF ESSEX
DEPARTMENT OF CHEMISTRY

LECTURESHIP IN AEROSOL SCIENCE

A special grant by the UGC enables the University to develop significantly its work in the field of aerosol science and an appointment has been made to a Readership in this field.

Applications are now invited for a second post, that of Lecturer (salary scale £7,190 — £14,125), for a period of five years, with a possibility of renewal, tenable from 1st October 1984 or as soon as possible thereafter. The main duties of the post will be to undertake research and postgraduate teaching in aerosol science and to contribute to undergraduate teaching in this and related areas. Applications are invited from candidates with experience in aerosol science or with an appropriate background and wishing to develop their interests in aerosol science research.

Applications (ten copies), including a curriculum vitae and the names and addresses of two referees, should reach the Registrar (AG/176/N), University of Essex, Wivenhoe Park, Colchester CO4 3SQ, from whom further particulars may be obtained, by 21 June 1984. (2156)A

MOLECULAR BIOLOGIST position for research scientist/assistant professor of medicine to participate in ongoing projects and develop independent research using recombinant DNA technology. PhD in biochemistry, molecular biology, or related area required. Postdoctoral experience preferred. Send resumé, description of research interests, and three letters of reference by 15 June 1984 to: Dr Ron MacQuarrie, Department of Chemistry, University of Missouri-Kansas City, Kansas City, Missouri 64110. The University of Missouri is an Equal Opportunity/Affirmative Action Employer. (NW574)A

UNIVERSITY OF ZIMBABWE

Applications are invited for the following posts:

LECTURESHIP/ SENIOR LECTURESHIP/ ASSOCIATE PROFESSORSHIP:

DEPARTMENT OF GEOLOGY

This post requires someone with an expert knowledge of metamorphic petrology, preferably with a PhD. An interest in economic geology would be an advantage, while experience in Electron-microprobe and/or analytical techniques are also desirable. The successful candidate will be required to teach metamorphic petrology in the undergraduate BSc General courses; to teach, and supervise field projects, at BSc Sepcial Honours level; and to supervise higher degree students as necessary. He/She may be required to teach introductory courses in the BSc General degree, and service in other Faculties.

LECTURESHIP/ SENIOR LECTURESHIP/ ASSOCIATE PROFESSORSHIP:

DEPARTMENT OF BIOCHEMISTRY (FOOD CHEMISTRY/ BIOCHEMISTRY POST)

Applicants should have a postgraduate degree, preferably a PhD in Food Science from a recognised university with teaching and research experience in a University Food Science programme The successful candidate will be expected to undertake teaching and research in Food Biochemistry and Food Analysis and to supervise MSc Projects in liaison with industry.

Salary scales: Lecturer: Z$10,440 x 456-$16,368 x 504-$17,376. Senior Lecturer: Z$17,880 x 504-$20,400. Associate Professorship: Z$18,384 x 504-$20,904.

Appointment on above scales according to qualifications and experience.

Conditions of service: Both permanent and short-term contracts are offered. Persons who are not Zimbabwean citizens may be appointed only on a short-term contract basis with an intial contract period of two years. Short-term contracts may, in exceptional cases, be extended.

Application forms and further particulars on the above posts can be obtained from the Director, Appointments and Personel, University of Zimbabwe, PO Box MP 167, Mount Pleasant, Harare, Zimbabwe, or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Closing date for receipt of completed application forms is 13 July 1984. (2208)A

POSTDOCTORAL ASSOCIATE position immediately available for recent PhD biochemist to participate in research program on control mechanisms for cell growth. Focus on the molecular signals controlling transcription and translation of cardiac proteins. Expertise in nucleic acid and protein chemistry essential. Experience in purification and identification of peptides and cell free translation of mRNA important. This is a collaborative program between the Department of Surgery of Yale Medical School and the Department of Biology, Yale University. Salary will be above NIH guidelines. Send curriculum vitae to: Graeme L Hammond, MD, Department of Surgery, Yale Medical School, PO Box 3333, New Haven, Conn 06510. Equal Opportunity Employer. (NW562)A

SEMINARS and SYMPOSIA

UNIVERSITY OF SURREY
DEPARTMENT OF MICROBIOLOGY
ONE DAY MEETING ON MYCOBACTERIAL INFECTIONS
Wednesday 6th June, 1984.

Innate & Acquired Resistance — Immunodiagnosis — Immunisation — Control.

Speakers: Dr J Stanford, Dr G Rook, Dr L Poulter, Dr C Brown, Dr J Brown, Dr J Colston.

Further details: D J Langley, Microbiology, University of Surrey, Guildford, GU2 5XH. (2119)M

ANTIBODY BINDING SITES AND THEIR EXPLOITATION IN SUBCELLULAR STUDIES
Guildford, 3-6 September 1984
Co-sponsor: U.S. Army Medical R & D Command

Immunological 'tools' will feature along with site-structure investigation at the 9th International Subcellular Methodology Forum, on the University of Surrey's congenial campus (near London). Speakers include O.J. Bjerrum, M.J. Crumpton, A.B. Edmundson, B.F. Erlanger, S. Hakomori, E. Kabat, K.L. Knight, P. Marrack, N.A. Mitchison, M. Raff, A.D. Strosberg, M. Waterfield and V.P. Whittaker. Topics include T-cell and neuronal receptors, the cytoskeleton, and neoplasia.

Contact Dr E. Reid, Guildford Academic Associates, 72 The Chase, Guildford GU2 5UL, U.K. (tel 0483-65324; telex 859643 PAYDAY G). (2195)M

WORKSHOP

WORKSHOP ON ABNORMAL CELLS, NEW PRODUCTS, AND RISK
Co-sponsored by:
FDA, NCI, NIAID, NHLBI, and CDC
will be held on July 30-31, 1984
at the NIH in Bethesda, MD, USA

The meeting has been organized to review issues and data relating to the use of abnormal cells in the production of drugs and biologics. Particular attention will be focused on three areas: (1) residual cellular DNA in products; (2) viruses endogenous to the cell substrate; and (3) residual cellular proteins in products. Discussions will follow the presentation of data, and there will be an attempt to develop consensus in each of the three areas during the afternoon of the second day. The Public Health Service Agencies co-sponsering this workshop believe it will assist them in making decisions on the support of research, the regulation of products, and the use of products derived from abnormal mammalian cells. The meeting is open to the public; however, individuals planning to attend should send their name and address to: Mary Ann Tourault, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20205 so that additional information can be sent in advance of the meeting. (NW575)V

STUDENTSHIPS

UNIVERSITY OF STRATHCLYDE
DEPARTMENT OF PHYSICS

DEVELOPMENT OF NOVEL LASERS AND INFRA-RED REMOTE SENSING

Applications are invited for three CASE Research studentships in Experimental Laser Physics. The projects will be concerned with the development of tunable solid state lasers for the visible and near infra-red regions and with the generation of ultra-short pulses, (co-operating body Barr and Stroud Ltd), development of tunable waveguide CO_2 lasers (co-operating body Edinburgh Instruments Ltd.), and with infra-red spectroscopy for remote sensing (co-operating body National Physical Laboratory).

Potential candidates, who should have or expect to obtain a good Honours Degree in Physics, Chemistry or Electronics can obtain further information from
Professor B. Henderson, Department of Physics, University of Strathclyde, John Anderson Building, 107 Rottenrow, Glasgow G4 0NG (2218)F

Prevention and Detection of Cancer

6TH INTERNATIONAL SYMPOSIUM

Vienna, Austria

26 to 29 November, 1984

SCIENTIFIC PROGRAM*

26 November, 1984

HOST FACTORS
- Familial host factors
- Genetic predisposition
- Aging and cancer
- Hormones and cancer
- Immune surveillance
- Psychosocial profiles and lifestyle

TOBACCO
- Behavioral changes
- Lung cancer
- Oral cancer
- Other cancer
- Ambient tobacco smoke
- Combination carcinogenic effect

MOLECULAR BIOLOGY I
- Oncogenes
- Biochemical mechanisms of carcinogenesis
- Virus and cancer

TOBACCO IMPLEMENTATION WORKSHOP
- Education
- Low tar cigarette
- Advertising (mass media)
- Taxation policy
- Agricultural alternatives
- Legislation

27 November, 1984

CLINICAL DETECTION
- Identification and surveillance of individuals at high risk exposure
- Identification of genetically susceptible individuals
- Biochemical markers of tumor diathesis and early cancer
- Cellular markers of tumor diathesis and early cancer
- Mass screening
- Automation in screening projects: use of high tech

IMMUNOPATHOLOGY
- Cell membrane structure and function
- Antibodies in diagnosis and surveillance of cancer patients
- Assessment of host immune response to tumors
- T cell subsets
- Tumor cell macrophage interactions
- Human hybridomas

MOLECULAR BIOLOGY II
- Chromosomes and cancer
- Differentiation and cancer
- Cytoskeletal alterations
- Cell kinetics and cancer

STRATEGIES FOR PREVENTION IMPLEMENTATION WORKSHOP
- Government organization
- Voluntary and community organization
- Medical and professional organization
- Individual habits and education

28 November, 1984

PRECURSORS
- Prenatal and childhood exposure to carcinogenic factors
- Genetic predisposition
- Clues from experimental carcinogenesis for prevention
- Biology of precursor lesions
- Identification of benign and potentially malignant lesions
- Specificity of environmental factors on precursor lesions

NUTRITION
- Vitamins
- Trace metals
- Protein and calories
- Fat
- Fiber
- Food additives and contaminants
- Childhood nutrition
- Epidemiology: micronutrients

VIROLOGY
- HTLV, leukemia and immunology
- HSV II and cervical cancer
- Papilloma virus and cervix
- EBV and human malignancies
- Hepatitis B and liver cancer

STRATEGIES FOR DETECTION IMPLEMENTATION WORKSHOP
- Government organization
- Voluntary and Community organization
- Medical: modalities to accomplish detection; cost benefit
- Individual: self-examination, self-questionnaire, motivation
- Priorities for training

29 November, 1984

TUMOR MARKERS
- Biologic basis
- Risk assessment
- Early cancer detection
- Detection of metastasis and recurrence
- Clinical guidelines
- Nuclear magnetic resonance

ENDOCRINE AND IDIOPATHIC CARCINOGENESIS
- Female genital tract
- Breast cancer
- Hepatic metabolism of steroids
- Thyroid cancer
- Prostatic and testicular cancer

CULTURAL PRACTICES
- Origins and reasons
- Difficulties of change
- Education in disease prevention: social impact
- Role of mass media
- Education in school
- Legislation

ENVIRONMENTAL INTERRELATIONS IMPLEMENTATION WORKSHOP
- Lessons from β-naphthalene
- Asbestos
- Agrochemicals (including agent orange example)
- Food stuffs (natural, artificial, Bracken fern, aflatoxins
- Air pollution
- Radiation pollution

ISPO Suite 303 207 East 85th Street New York, N.Y. 10028 USA

(NW556)M

*CME credit hours in Category I

Sponsor
International Society for Preventive Oncology
Co Sponsors
The World Health Organization
Austrian Cancer Society/Austrian Cancer League
Association of Clinical Scientists – USA

SCIENTIFIC PROGRAM

The program includes overview lectures, panels, poster sessions, scientific exhibits and special workshops. Reports will be presented on recent progress in multifactorial etiology of oncogenesis, molecular biology, identification of high-risk groups, tumor susceptibility, and clinical and laboratory manifestations of cancer, including tumor markers. Participants include the clinician, epidemiologist, pathologist, experimental oncologist, immunologist, socioeconomist, educator and members of the health care team.

IMPLEMENTATION WORKSHOPS

Special workshops are designed for critical appraisal of current data. Discussions will concentrate on actions to be taken and on the implementation of existing knowledge for effective cancer control by primary and secondary prevention.

PANELS ON CLINICAL ONCOLOGY OF SPECIFIC SITES

Daily panel topics include:

- epidemiology
- carcinogenic factors
- predisposing factors
- precancerous conditions
- markers for risk assessment
- detection and diagnosis by cytology, endoscopy, radiology, imaging, enzymology, immunology, ultrasound, histology, and other modalities
- management following detection
- prognostic determinants
- prevention

FACULTY / PARTIAL LIST

P BANNASCH	(FRG)
JG BEKESI	(USA)
E BJELKE	(NORWAY)
G BLAUDIN DE THE	(FRANCE)
RR BRENTANI	(BRAZIL)
RD BULBROOK	(ENGLAND)
B CZERNOBILSKY	(ISRAEL)
F DEO	(INDIA)
G DHOM	(FRG)
S ECKHARDT	(HUNGARY)
A ENGLUND	(SWEDEN)
MY FELDMAN	(ISRAEL)
B FISHER	(USA)
AL FRANK	(USA)
R GALLO	(USA)
SA GELLER	(USA)
RA GOOD	(USA)
NJ GRAY	(AUSTRALIA)
E GRUNDMANN	(FRG)
C HEDINGER	(SWITZERLAND)
T HIRAYAMA	(JAPAN)
P HOBBS	(ENGLAND)
JC HOLLAND	(USA)
JF HOLLAND	(USA)
JH HOLZNER	(AUSTRIA)
H ICHIKAWA	(JAPAN)
DJ JUSSAWALLA	(INDIA)
JV KLAVINS	(USA)
G KLEIN	(SWEDEN)
W KNAPP	(AUSTRIA)
M KUNZE	(AUSTRIA)
FP LI	(USA)
G MACHNIK	(DDR)
F MITELMAN	(SWEDEN)
V NAPALKOV	(USSR)
AM NEVILLE	(ENGLAND)
HE NIEBURGS	(USA)
R ROEMER	(USA)
J SCHLOM	(USA)
D SCHMAHL	(FRG)
K STANLEY	(WHO)
J STJERNSWARD	(WHO)
P STRAX	(USA)
SUN TSUNG-TANG	(CHINA PR)
G TODARO	(USA)
VEO VALLI	(CANADA)
IB WEINSTEIN	(USA)
GL WIED	(USA)
EL WYNDER	(USA)
A ZUCKERMAN	(ENGLAND)
H ZURHAUSEN	(FRG)

ISPO CANCER CONTROL AWARDS

ISPO is pleased to offer awards for original research that may lead to improved cancer control in:

- Tumor markers for risk assessment;
- Risk factors and their control in developing countries; and
- Tumor markers for detection of early neoplasms.

Applications are available upon request

GENERAL INFORMATION AVAILABLE

- Award applications
- Symposium brochure
- Accompanying persons program
- Special airfares
- Tours to Prague, Budapest, Salzburg
- Opera, concert and seasonal celebrations
- ISPO membership information

CALL FOR ABSTRACTS

Abstracts are invited for poster sessions and podium presentations on program-related topics. Deadline for receipt of abstracts: JULY 23, 1984.

INQUIRIES BY MAIL

International Society for Preventive Oncology
207 East 85th Street, Suite 303
New York, NY 10028, USA

INQUIRIES BY PHONE:
Toll-free in USA: 1-800-527-0297
Outside Europe:
(usa-214) 392-3663
In Europe: (austria43-222) 52-0544

FELLOWSHIPS

E M B O
European Molecular Biology Organization
LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY AUTUMN 1984 AWARDS
Next deadline: August 15, 1984

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligibile a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are awarded initially for one year, but depending on a review of progress, they be renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is August 15, 1984. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on October 29, 1984.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F.R. Germany. (W1016)E

Clare Hall, Cambridge

Research Fellowships in Physical and Biological Sciences

The Governing Body of Clare Hill proposes to elect two or more non-stipendiary Research Fellows for a period of three years, starting not earlier than October 1984, to work in mathematics, the physical or biological sciences, or technology. There are no restrictions on age or sex.

The closing date for applications is 14th July 1984.

Application forms may be obtained from Clare Hall, Herschel Road, Cambridge (Tel. Cambridge 63330). (2164E)E

UNIVERSITY OF ABERDEEN

INSTITUTE OF ENVIRONMENTAL AND OFFSHORE MEDICINE

POSTDOCTORAL FELLOWSHIP

Applications are invited from suitably qualified graduates for a one-year appointment to the Institute. The work will involve investigation into the incidence of decompression sickness in commercial divers following air-diving in the UK sector of the North Sea with a view to identifying factors which might contribute to the condition. Applicants should have a working knowledge of diving techniques. Statistical/epidemiological experience would be an advantage. Further details available from Dr T Shields, Tel 0224 684432.

Salary on the Range IA scale for Research and Analogous Staff, £7,190 — £11,615 per annum (under review) with appropriate placing.

Application forms and further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 30 June 1984. (2187)E

Please mention

nature

when replying to these advertisements

THE DIABETIC ASSOCIATION
R.D. LAWRENCE RESEARCH FELLOWSHIPS

The Association seeks applications from graduate scientists or medically qualified persons working in the field of diabetes mellitus for the above Fellowships established in memory of Dr. R.D. Lawrence, the founder of the British Diabetic Association.

The Fellowships are for two years and the salary will be paid at registrar or equivalent university scales.

The closing date for applications is 25th June 1984 and applicants must be available for interview on 4th July 1984.

Forms available from: The Assistant Secretary, The British Diabetic Association, 10, Queen Anne Street, London, W1M 0BD. Tel: 01-323 1531. (2200)E

VICTORIA UNIVERSITY OF WELLINGTON
New Zealand

UGC POSTDOCTORAL FELLOWSHIPS

Applications are invited from suitably qualified persons who are no more than 35 years of age. Priority will be given to awards in current growth research areas in science in this country, and applications are invited in the following areas: Biochemistry, Botany, Chemistry, Computer Science, Geography, Geology, Geophysics, Mathematics, Operations Research/Statistics, Physics, Psychology, Zoology.

Conditions of Appointment and method of applications are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Administrative Assistant (Appointments), Victoria University of Wellington, Private Bag, Wellington, New Zealand, with whom applications definitely close on 16 July 1984. (2207)E

UNIVERSITY OF ROCHESTER

NEUROBIOLOGY OF AGING TRAINING

for M.D's and Ph.D's. Several fellowships, supported by the National Institute on Aging, are now available for Ph.D's and post-residency M.D.'s who are interested in research training in the neurobiology of ageing. We particularly encourage applicants with diverse backgrounds who wish to apply their past training to problems of the aging CNS. Such backgrounds include molecular biology, biochemistry, pharmacology, neurology, psychiatry, medicine, gerontology, neural sciences, and others. The training program offers an intense research apprenticeship, brief circulation through a variety of laboratories, seminars, and clinical correlations. Twenty potential preceptors are in the Departments of Anatomy, Brain Research, Medicine, Neurology, and Psychology.

Apply to: **Dr Paul D. Coleman, Department of Anatomy, University of Rochester, Medical Center, Rochester, N.Y. 14642.** (NW550)E

POSTDOCTORAL FELLOWSHIP — Research Associate to study molecular cloning and genetic engineering with animal tissues or plant cells. Send cv to Dr J P Thirion, Microbiology Department, Faculty of Medicine, Sherbrooke, P Que, Canada J1H 5N4. (NW563)E

ASSISTANTSHIPS

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY
Biophysics Section

DEPARTMENT OF PHYSICS

RESEARCH ASSISTANTSHIPS IN PROTEIN CRYSTALLOGRAPHY

Post-Doctoral Research Assistantships are available on a programme of research into protein structure by X-ray crystallography. An initial appointment can be made for up to three years, but further extension is likely. Two posts are available. One requires an experienced protein crystallographer: the other would suit a young PhD with a knowledge of crystallography.

Apply as soon as possible to Prof D M Blow, RS, Blackett Laboratory, Imperial College, London SW7 2BZ, including a brief CV and the names of two referees. (2183)P

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Postdoctoral Research Assistantship to work on the isolation and reconstitution of brain D_2 dopamine receptors. The project is funded by MRC and is initially for one year (starting from October 1st, 1984), renewable for a further two years. The salary is on the 1A scale starting at £7,190 per annum.

Applicants should send a curriculum vitae including the names of three referees to Dr P G Strange, Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, as soon as possible. (2154)P

CONFERENCES and COURSES

NATO ADVANCED STUDY INSTITUTE

Radiation Carcinogenesis and DNA Alterations Corfu, Greece October 7-20, 1984

A series of lectures, tutorials and panel discussions will focus on how radiation-induced DNA alterations might be involved in radiation carcinogenesis. Topics will include activation of oncogenes, mutational alterations, chromosomal rearrangements and the possible role of DNA repair. Carcinogenesis in animals and *in vitro* transformation will be discussed as well as model implications for quantitative human risk assessment.

Faculty includes: Dr R.E. Albert, N.Y., U.S.A.; Dr A. Balmain, Glasgow, U.K.; Dr G.W. Barendsen, Rijswijk, N.L.; Dr J. Broerse, Rijswijk, N.L.; Dr F.J. Burns, N.Y., U.S.A.; Dr P. Cerutti, Lausanne, Switzerland; Dr K.H. Chadwick, Wageningen, U.K.; Dr S.B. Field, London, U.K.; Dr E.M. Fielden, Harwell, U.K.; Dr A.H. Kellerer, Wurzburg, D.R.F.; Dr J.B. Little, Boston, U.S.A.; Dr M. Paterson, Chalk River, Canada; Dr A. Rauth, Toronto, Canada; Dr G. Silini, Vienna, Austria; Dr I.P. Sinclair, New Jersey, U.S.A.; Dr K.C. Smith, California, U.S.A.; and Dr A.C. Upton, N.Y., U.S.A.

The course is open to scientists and postdoctoral workers in cell and molecular biology, radiation biology or biochemistry. Assistance to defray living costs, etc. will be available.

For further information contact: Dr F.J. Burns, NYU Department of Environmental Medicine, 550 First Ave., New York, N.Y. 10016. (NW564)C

UNIVERSITY OF ST ANDREWS
MSc Course in Radiation Biophysics

This taught course occupies a calendar year with a 6 month formal component and a 6 month research project.

The course is interdisciplinary and run jointly by the Departments of Anatomy and Experimental Pathology and Physics. It is structured to accommodate those qualified either in the physical sciences or the life sciences and caters for differing interests and career intentions of students. It is suitable, for example, for those considering careers in research with, and application and control of, radiation in science, industry, medicine and power production. Research Council scholarships (MRC) are available.

The course is that previously provided by the Department of Medical Biophysics at Dundee University.

Enquiries and applications to Dr A.C. Riches, Department of Anatomy and Experimental Pathology, University of St Andrews, St Andrews, Fife KY16 9TS. (2029)C

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UNIVERSITY OF GLASGOW
Institute of Physiology

RNIB RESEARCH STUDENTSHIP

Quantitative study of synapse formation in normal and amblyopic retinae re maturity of ganglion cell receptive field. Involves electron microscopy with scope for diversification. Excellent facilities: full training given. Good Honours degree in Physiology, Anatomy or related subject required. Start 1st October for 3 years: registration for PhD degree.

Applications, including 2 referees, to Dr J D Morrison, from whom further particulars are available (tel 041 339 8855 ext 552). (2169)C

UNIVERSITY OF EDINBURGH
DEPARTMENT OF METEOROLOGY

NERC STUDENTSHIP

Applications are invited for a Research Studentship on 'The Meteorological Influences on the Distribution of Rainfall Acidity in the U.K.' This is a case studentship with the Institute of Terrestrial Ecology, Edinburgh. Candidates should have or expect to obtain in 1984, a first or upper second class honours degree in a physical science or in environmental science. Further details can be obtained from Dr K J Weston, Department of Meteorology, University of Edinburgh, Edinburgh EH9 3JZ. (2216)F

STUDENTSHIPS

Portsmouth Polytechnic
DEPARTMENT OF BIOLOGICAL SCIENCES

1. Research Assistantship
Applications invited for a ROSCM funded post to investigate marine microbial fouling of ship.
Salary scale: £4,515 – £5,649 per annum (under review)

2. One SERC Studentship:
a) Ultrastructural and immunological studies of developing stages of nematode parasites
b) Microbial deterioration of timber
c) An investigation of post germination attachment in some common marine fouling algae.

Closing date for the above posts 18th June 1984.

3. MSc Course in Biodeterioration of Materials
SERC Advanced Course studentships available for suitably qualified students.

Application forms and further details for (1) from the Personnel Office, Nuffield Centre, St Michaels Road, Portsmouth. Tel: (0705) 825451. Ref: B

Further details for (2) and (3) from Administrative Assistant, Department of Biological Sciences, Portsmouth Polytechnic, King Henry 1 Street, Portsmouth. (2213)F

UNIVERSITY OF NOTTINGHAM
and
TROPICAL DEVELOPMENT RESEARCH INSTITUTE

RESEARCH STUDENTSHIP
Stability of tropical fish muscle proteins

Applications are invited from those holding a first or second class honours degree in food science, chemistry, biochemistry or related discipline for a TDRI research studentship tenable in the Department of Applied Biochemistry and Food Science. The successful candidate will be required to register for a higher degree of the University of Nottingham and will be engaged full-time on a programme designed to evaluate the factors affecting the stability of tropical fish muscle proteins during processing.

The scholarship is for 3 years.

Applicants should forward a curriculum vitae including the names of two referees to:- Dr R G Bardsley, Dept of Applied Biochemistry & Food Science, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics LE12 5RD. (Telephone Kegworth 2386) (2179)F

TRENT POLYTECHNIC
DEPARTMENT OF LIFE SCIENCES

SERC CASE STUDENTSHIP

Applications are invited for the above studentship. The project, in collaboration with Bush Boake Allen Ltd, London, will investigate the use of microorganisms or enzymes extracted from them in the production of fragrances and flavours.

Applicants should hold or expect to hold a first or upper second class honours degree in biochemistry, microbiology, biotechnology or related subjects.

Further details and form of application are available from the Staff Officer, Trent Polytechnic, Burton Street, Nottingham NG1 4BU. Closing date: 18 June 1984. (2155)F

THE UNIVERSITY OF LEEDS
DEPARTMENT OF PLANT SCIENCES

RESEARCH STUDENTSHIP

Applications are invited for the above postgraduate studentship from final undergraduates or from recent graduates with a good honours degree in the agricultural sciences or in plant biology. The student will work in a group funded by ICI to study the growth and physiology of winter barley and will be registered for a higher degree on a full-time basis.

Further details can be obtained from Professor Elston, Department of Plant Sciences, The University, Leeds LS2 9JT, (tel (0532) 431751 Ext 377) to whom applications, with the names and addresses of two referees, should be submitted as soon as possible. (2160)F

NUTRITIONAL CONSULTATIVE PANEL
of the
UK Dairy Industry
is offering a

POST GRADUATE STUDENTSHIP

for work, possibly leading to a higher degree in a British University or Research Institution on a Nutritional subject related to: Milk and Milk Products in the Human Diet. Examples of subjects which the Panel has identified are:
1) the role of calcium in cow's milk
2) the role of cow's in the weaning of human infants.

Other subjects will be considered. Salary will follow MRC guidelines. Apply in writing, maximum 500 words, before 22nd June 1984 to: The Secretary, Nutritional Consultative Panel, Giggs Hill Green, Thames Ditton, Surrey KT7 0EL, including proposed department where work will be carried out, curriculum vitae and names and addresses of 2 referees. (2182)F

STUDENTSHIPS

UNIVERSITY OF BATH

DEPARTMENT OF BIOCHEMISTRY

Students who have or are expecting to obtain, an upper second class degree are invited to apply for this

SERC STUDENTSHIP

The topic of research is the Molecular biology of dormancy in plants. The involvement of gene expression in the maintenance and breaking of dormancy will be studied. Purification of some of the enzymes of carbohydrate metabolism and their messenger RNA's will be undertaken with the ultimate aim of inserting cDNA copies into various plasmid vectors. It is expected that this work will give insight into the regulation and control of dormancy.

Applications with a curriculum vitae and the names of two referees should be sent to Dr P P Rutherford, Biochemistry Department, 4 West University of Bath Bath BA2 7AY. (2197)F

UNIVERSITY OF YORK

DEPARTMENT OF BIOLOGY

Applications are invited for a postgraduate studentship leading to a DPhil to study either immunological mechanisms in chronic relapsing experimental allergic encephalomyelitis, an animal model for multiple sclerosis or the effects of neurotropic virus infections on defined populations of central nervous system cells in culture. Candidates must have (or expect to gain) a First or Upper Second class degree. The studentship is funded by the Multiple Sclerosis Research Fund Ltd. on terms and conditions similar to Research Council studentships. For further information contact Dr M G Rumsby or Dr A J Suckling in the Department of Biology, University of York, Heslington, York YO1 5DD. (2221)F

QUEEN MARY COLLEGE
(University of London)

School of Biological Sciences

NERC RESEARCH STUDENTSHIPS

Two NERC Research Studentships are available for postgraduate research on the following topics:

(1) Reproductive investment and transmission success in helminth life cycles;

(2) Trace metal detoxification in crustaceans: separate or integrated systems?

Topic (1) will be supervised by Dr R C Tinsley; topic (2) by Dr P S Rainbow.

Applications should be sent to the relevant supervisor by 14th June, at the following address: School of Biological Sciences, Queen Mary College, Mile End Road, London, E1 4NS. (2205)F

UNIVERSITY OF EDINBURGH

DEPARTMENT OF GENETICS

A POSTDOCTORAL/ RESEARCH ASSOCIATE

vacancy exists for a scientist with experience of recombinant DNA/DNA sequencing. The work will be concerned with a study of host/virus nucelotide sequence homologies. The post is available from 1st July 1984, and is grant supported until 30th September 1985 in the first instance. Salary according to age and experience on academic scale £6,310 to £11,615.

Further particulars available. Applications with cv and names and addresses of two referees should be addressed to the Secretary's Office, Old College, South Bridge, Edinburgh.

Please quote reference No. 5175. (2226)F

THE UNIVERSITY OF LEEDS

DEPARTMENT OF CARDIOVASCULAR STUDIES

MRC STUDENTSHIP

Applications are invited for an MRC studentship commencing on 1 October 1984. The project is to investigate the convergence of cardiac and baroreceptor afferent fibres onto single neurones in the modulla oblongata.

Applicants should possess or expect to obtain a first class or upper second class degree in Physiology or a related discipline.

Applications, including the names of two academic referees, should be submitted to Dr P E McWilliam, Department of Cardiovascular Studies, University of Leeds, Leeds LS2 9JT. (2225)F

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF BIOCHEMISTRY

SERC POSTGRADUATE STUDENTSHIP IN BACTERIAL MOLECULAR BIOLOGY

Applications are invited for research on the molecular biology of either (i) RNA polymerase (ii) the multivalent vitamin B_{12} receptor, or (iii) methionine biosynthesis in Escherichia coli.

The studentship will commence in October 1984; registration is initially for an M Phil.

Applicants possessing a 2(i) or higher in an appropriate degree should send a curriculum vitae and the names of two referees to Dr Robert E Glass, Department of Biochemistry, Queen's Medical Centre, Nottingham NG7 2UH (Telephone: 0602-700111 ext 3395) from whom further information may be obtained. (2153)F

EAST MALLING RESEARCH STATION

FRUITERERS STUDENTSHIP

Applications are invited for a postgraduate studentship award by the Worshipful Company of Fruiterers for research into factors which affect eating quality of apple fruits produced by modern growing methods.

Applicants should have, or expect to obtain, a First or Upper Second class honours degree in botany, horticulture or a related subject, and must be able to commence the three year studentship in August 1984 at the latest.

Application form and further details from the Deputy Secretary, East Malling Research Station, Maidstone, Kent ME19 6BJ.

Closing date for applications is 15 June, 1984. (2185)F

KING'S COLLEGE LONDON
(University of London)

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a SERC-CASE post-graduate studentship in association with Ciba-Geigy Pharmaceuticals Division, Horsham. The project will involve studies on the properties and regulation of phospholipase C in human blood platelets with especial reference to phosphoinositide metabolism.

Applicants should have, or expect to obtain, a First or Upper Second Class Honours degree in Biochemistry or a relevant related discipline.

Applications, including the names and addresses of two referees, should be sent to Professor M C Scrutton, Department of Biochemistry, King's College London, Strand, London WC2 2LS to arrive as soon as possible, preferably before June 7 1984. (2171)F

GEORGE HENRY LEWES STUDENTSHIP

Applications are invited for this Studentship to pursue original investigation in Physiology. Appointment may be from 1 October 1984 for one year with a possibility of renewal for up to two further years. The award will provide an annual maintenance grant in line with the maintenance element of a Research Council research studentship; and the Trustees will pay University and College fees for the holder at the rates charged to science research students from the UK.

Candidates should by 16 July 1984 send (a) a statement of their qualifications and their need for pecuniary help, (b) the subject of their proposed research, and (c) the names and addresses of two referees, to Professor R D Keynes, Physiological Laboratory, Downing Street, Cambridge CB2 3EG, from whom further particulars may be obtained. (2217)F

REGIONAL NEUROLOGICAL CENTRE
Newcastle General Hospital

DEPARTMENT OF SURGERY (NEUROSURGERY)

EXPERIMENTAL STROKE
PhD Studentship
(Junior Research Associate)

Applications are invited from suitably qualified candidates (1 or 2.1 in physiology or biochemistry) for the above post, which is funded for 3 years by an MRC Project Grant.

Mechanisms of loss of neurological function in acute stroke will be examined: the studies will provide experience of a range of acute experimental techniques, and an opportunity to develop appropriate topographical biochemical methods.

Further information may be obtained from Mr A J Strong, Regional Neurological Centre, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE, tel Newcastle 738811, ext 647. Closing date for applications (to include cv and names of 2 referees) 18th June. (2222)F

QUEEN ELIZABETH COLLEGE
Kensington
(University of London)

DEPARTMENT OF PHYSIOLOGY

PHD STUDENTSHIP

Applications are invited for a Research Studentship in the Department of Physiology, tenable for three years commencing October 1984. The successful candidate will work with Dr G E Mann on the hormonal regulation of membrane transport in the normal and diabetic pancreas. The study will involve assessing the influence of islet (insulin, glucagon and gastrointestinal hormones or rapid amino acid uptake by the exocrine epithelium.

The successful applicant would be expected to register for a higher Degree. Candidates should possess or expect to obtain, a First or Upper Second Class Degree in Physiology Biochemistry or a related subject.

The Department has excellent research facilities for the above project and other closely related topics. Research and teaching will be strengthened by the merger of Queen Elizabeth College and Chelsea College with King's College London.

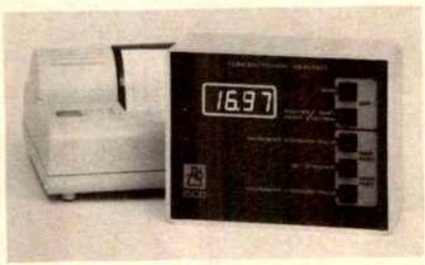
Applicants should apply in writing including their curriculum vitae and the names of two referees to Professor D L Yudilevich, MD, Department of Physiology, Queen Elizabeth College, University of London, Campden Hill Road, London W8 7AH. (2203)F

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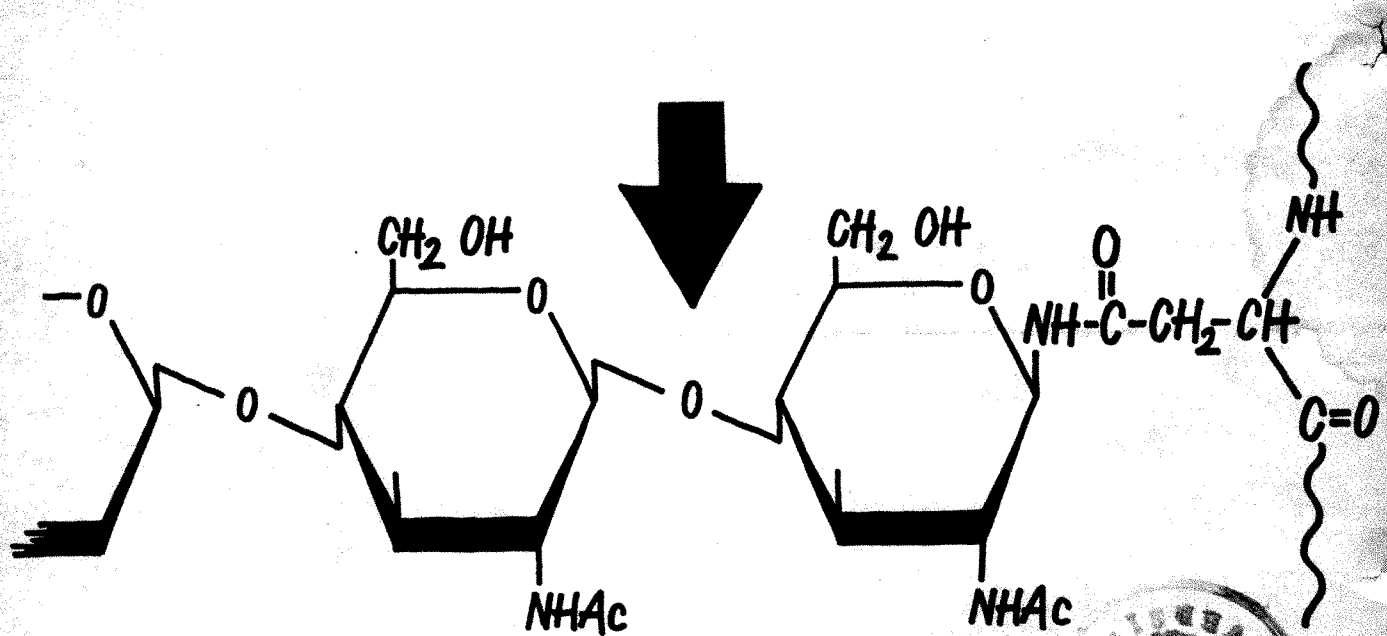
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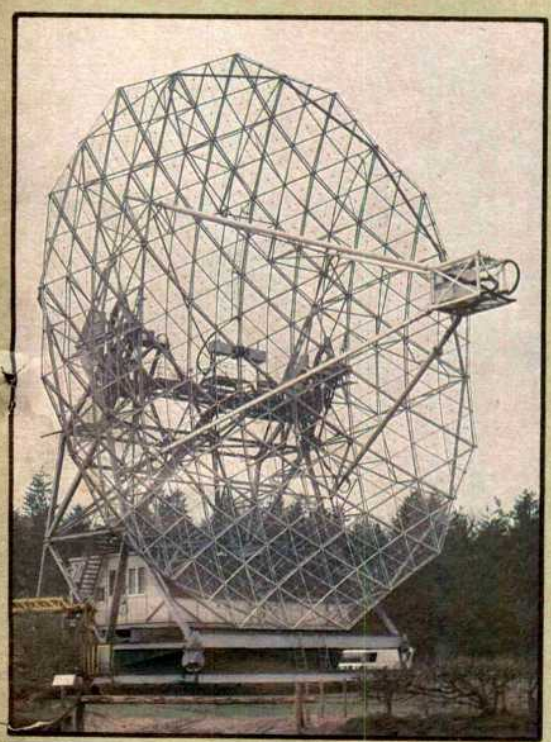
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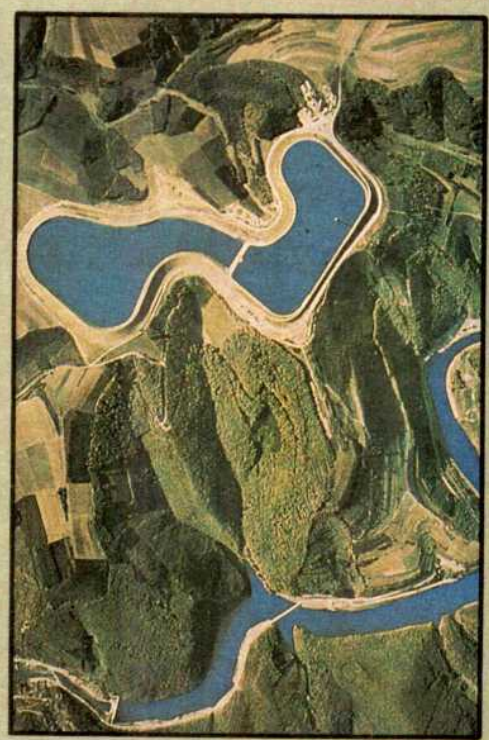
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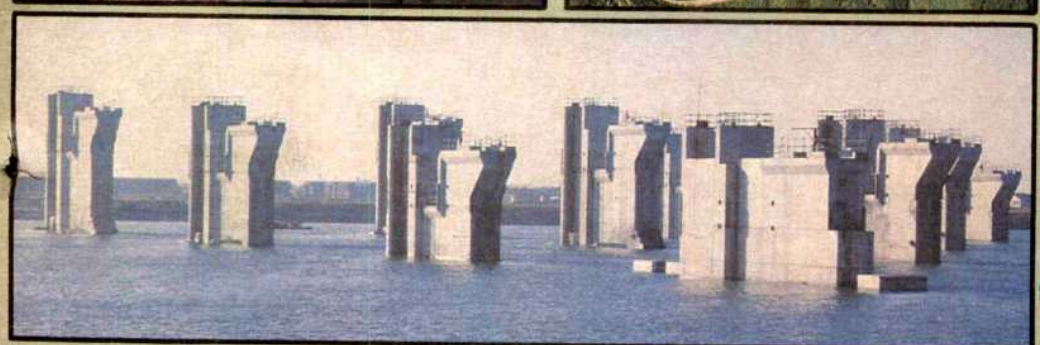
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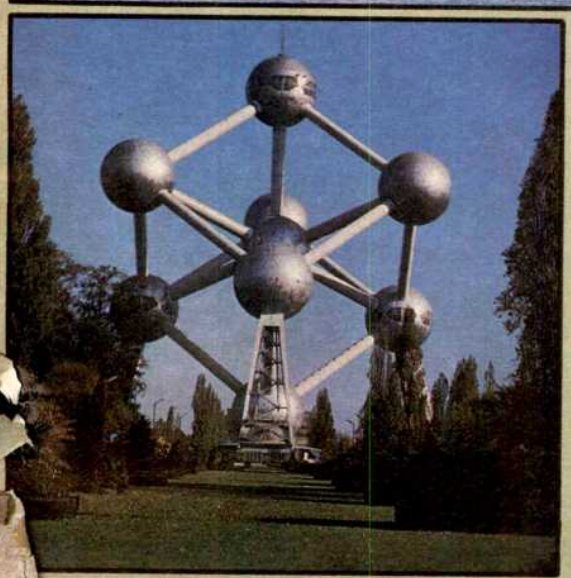
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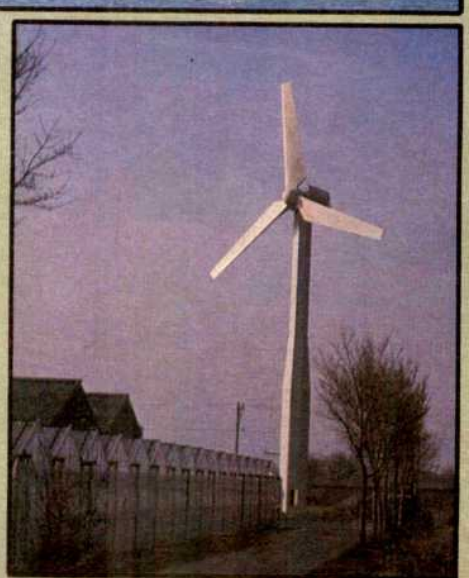






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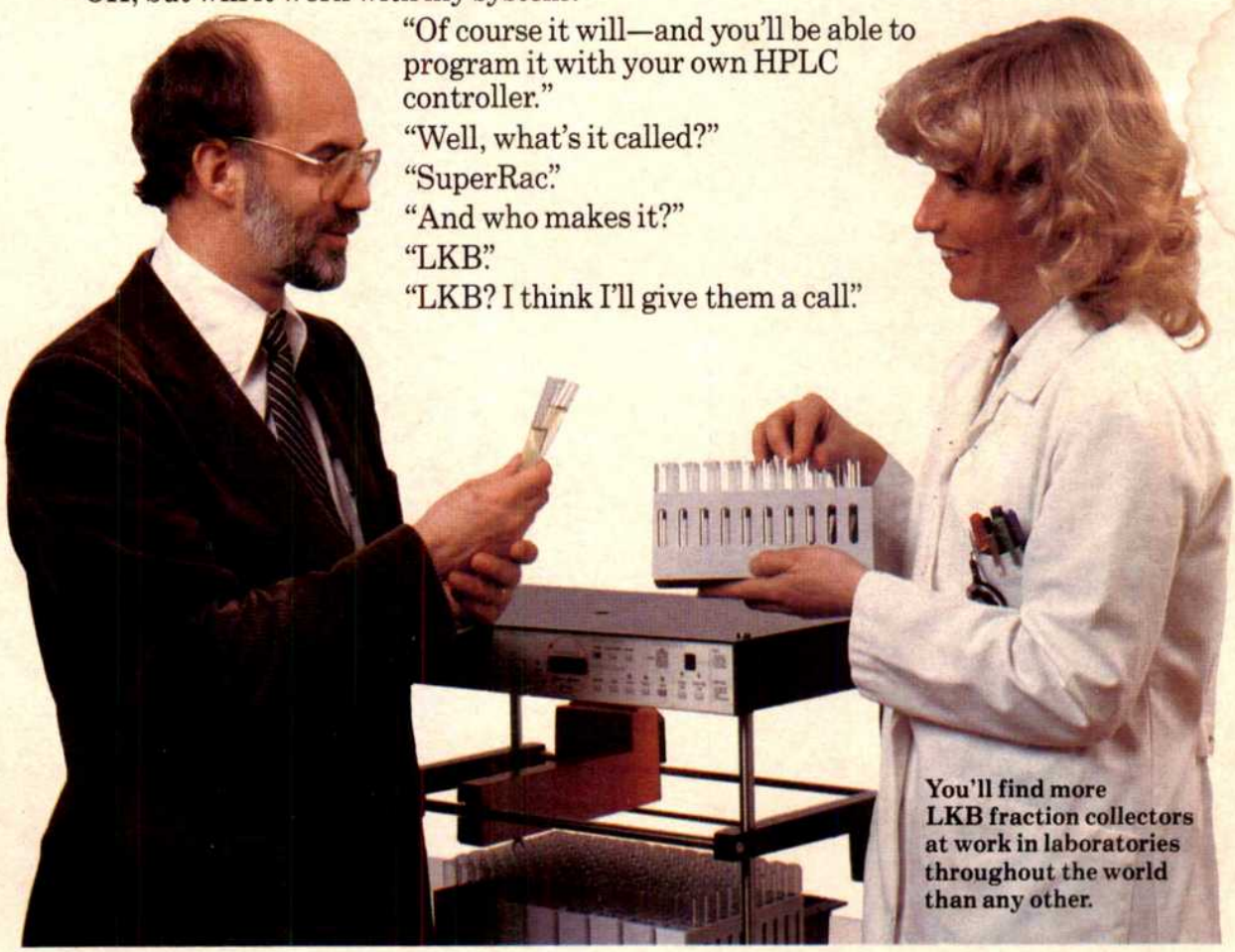
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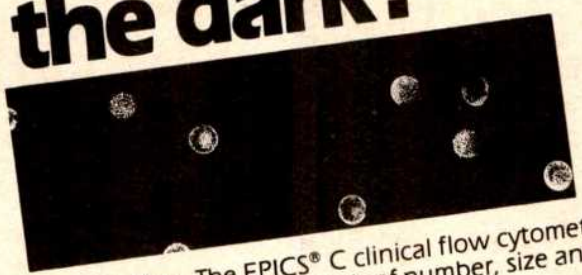
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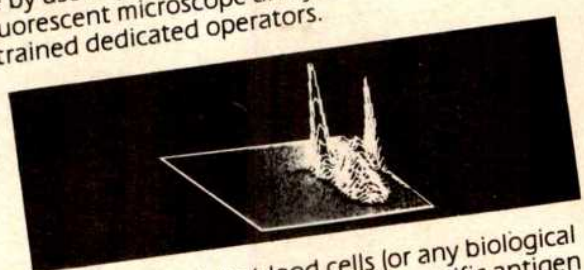
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CONFERENCE DINNER: The Conference dinner will be held on Tuesday evening, September 11th, in the Imperial Ballroom. TO INSURE SEATING AT THE CONFERENCE DINNER, please include your dinner ticket requirements and payment of $25 per person with your Registration.

POSTER SESSIONS: A limited space for posters will be made available. Please write to: Poster Sessions Editor, Nature Publishing Company, 15 E 26 St., N.Y., NY 10010.

RECRUITMENT CENTER: Private meeting rooms and staff will be provided for recruitment executives and scientists to discuss employment opportunities. Contact: Miss Gene Fein, Recruitment Services Manager, Nature Publishing Co., 15 E. 26 St., N.Y., NY 10010, (212) 689-5900.

THE MOLECULAR BIOLOGY OF

CANCER

Conference Program
September 10-12, 1984
Boston Park Plaza Hotel

Daily Themes:

DAY 1 - ONCOGENES,
DAY 2 - THE CANCER CELL,
DAY 3 - PROSPECTS/PROBLEMS.

DAY 1

MONDAY, 10 September
Exhibition Viewing: 8:30am— 6:30pm
Recruitment Center: 9:30am— 6:00pm

ONCOGENES AND GROWTH FACTORS — WHAT THEY ARE AND WHAT THEY DO

9:00— 9:15	Chairman
9:15—10:00	Oncogenes *R. Weinberg (MIT)*
10:00—10:45	Growth factors *M. Sporn (NIH)*
11:15—12:00	HTLV *R. Gallo (NIH)*
12:00—12:30	Clinician's View *S. Broder (NIH)*
2:00— 4:00	Roundtables with morning speakers
4:30— 5:30	The Cancer Cell *J. Cairns (Harvard)*

DAY 2

TUESDAY, 11 September
Exhibition Viewing: 8:30am— 6:30pm
Recruitment Center: 8:30am— 6:00pm

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11:15—12:00	Carcinogens and promoters *I.B. Weinstein (Columbia)*
12:00—12:30	Clinician's View *F. Gilbert (Mt. Sinai)*
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9:15—10:00	Diagnostics and therapeutics *G. Todaro (Oncogen)*
10:00—10:45	Immunotoxins *J. Uhr (Texas)*
11:15—12:00	Drug resistance *A. Varshavsky (MIT)*
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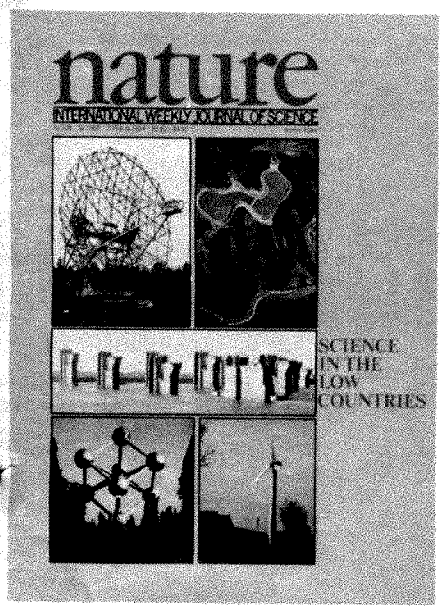
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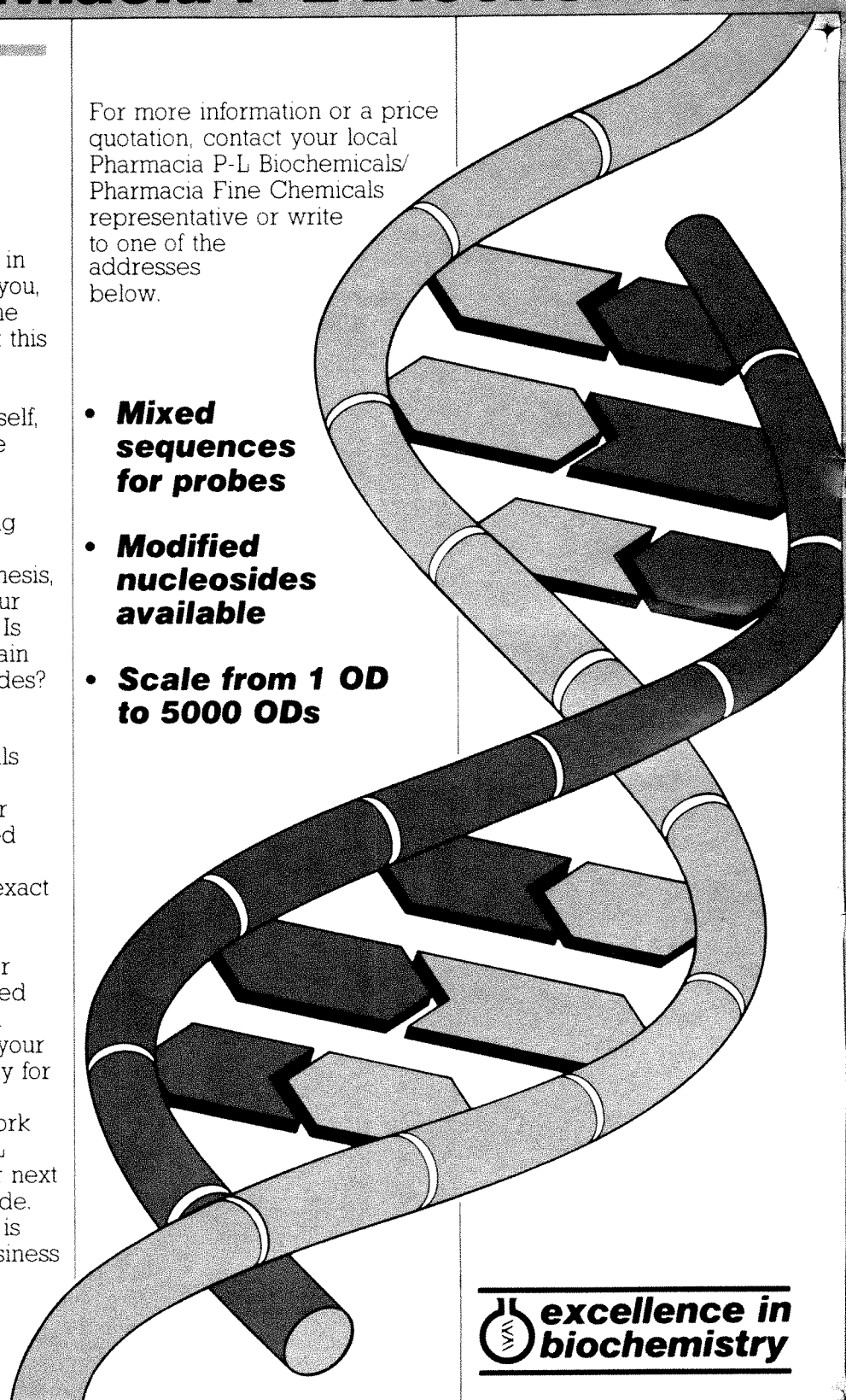
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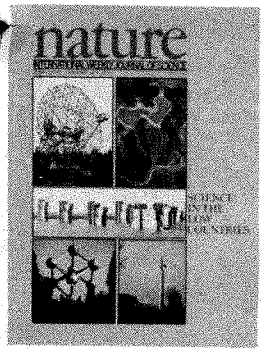
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nature

Revivifying Europe's research

The next European summit at Fontainebleau could be another dog-fight, spend time making speeches about European federation or — more prudently — find a middle course.

EUROPE may not be made in the few weeks ahead, but there is a danger that it could be broken. And certainly the month that lies ahead will be crucial for the whole enterprise. First come the European elections, only the second occasion on which representatives to the European Parliament have been chosen democratically and not simply nominated by their cronies. Then follows the next meeting of European political leaders at Fontainebleau, formally an occasion when the rest of Europe might thank President François Mitterrand of France for having been an energetic president of the Community (which would be true) but in practice more likely to be dominated by two quite different themes — the British Government's demand that it should be given a substantial rebate (say, £1,200 million) on the contributions it paid last year into the common budget, and the more general proposal that Europe, having made a bad job of creating a common market, should now go on to build a federal state. Between the ridiculous and the sublime, the serious opportunities at Fontainebleau are likely to be forgotten.

The survey of the condition of science in Belgium and the Netherlands which appears elsewhere in this issue points more accurately to some of the tasks that need to be attempted on a European scale (see p.491). Two of Europe's smallest states, in recent memory among the most prosperous, find themselves unexpectedly having to struggle to keep up with the times. As elsewhere, public budgets are being cut so as to escape inflation, the problems of catching up with the new technology are daunting and people worry that established institutions and ways of doing civil business may be damaged by too rapid a transition from the past to an uncertain future. In one way or another, the whole of Europe is similarly affected.

The calculation in the early 1950s was that if the common market that is the core of the Treaty of Rome could be made to function, the result would be an industrial population as large as any other in the world, prosperous and industrially creative. In the event, the common market has functioned, but only fitfully. In some respects, the arrangements have been an expensive joke (the butter mountain, the wine lake, and all that). In others (as at a host of national customs posts, which innocents may think should long since have disappeared), chauvinism has triumphed over legality. Public purchasing is still largely determined by public authorities, usually governments, in what seems to be the national interest. The result is that Europe is far from being the productive place the founding fathers foresaw. Those meeting at Fontainebleau should spend at least some time reminding themselves that their dream has not come true.

Chauvinism

Where they go from there depends on their estimate of what has gone wrong. To the extent that chauvinism is still an impediment to the efficient division of labour, it must be supposed that not all of the members of the common market have been serious all of the time. Nor are they now. The British Government is not the only political party whose representatives will be fighting next week's election on a platform which promises that national interests, general or particular, will be fiercely defended at Brussels. Even if these promises are not always kept in full, it is disappointing that they should be though necessary at all. The threatened fight at Fontainebleau over the British rebate could yet become the signal for the disintegration of the whole experiment.

The remedy, now as at the past two European summits (at Athens and Versailles) is that the participants should be patient, reckoning that their goal is a moving target, and that they should find other things to talk about than the issues on which they are most sharply divided. Here is a shopping list:

- Europe is alarmed at its technical inadequacies, for which reason the European Commission has been putting together programmes for collaborative research and development which must be transnational to attract support. But the Commission has put the cart before the horse. If the division of labour were economical (as one day it may be), industrial companies would shoulder responsibility for applied research, but Europe in general would have an important role to play in the support of more fundamental work, just as national governments now have. So why not begin in this neck of the woods, but slowly, recognizing that it will take time to weld Europe's academic and research community into a single entity, preserving its diversity the while?
- Europe is concerned that an efficient division of labour would leave some member states more prosperous than others in ways that would not automatically be offset by the migration of labour (formally made free under the existing rules, but inhibited by language and inertia). This fear is the seat of many of the restraints on the working of the common market now widely practised. But why not then follow the simple course, followed nationally in most of Europe, of arranging that the socially disadvantaged are somehow compensated from the well-being of others? The European social fund now administered from Brussels is too small to be significant and too discretionary to seem fair.
- Europe is by fits and starts scared stiff that it will once again become a battlefield, a danger made all the sharper by its knowledge that it is neither prosperous nor determined enough to defend itself. There can be no quick solution. The lack of resources is a more pressing restraint than the lack of will, while the problems of approaching the notion of a common defence would reawaken the arguments that made the 1950s wretched. But if the European Communities are to survive and to fulfil their promise, a start has to be made somewhere. Encouraging Europeans to join each others' armed services would be an interesting point, not much canvassed hitherto.

None of this quite lives up to President Mitterrand's eloquent espousal, in Strasbourg the other day, of the European Parliament's plan for a federal Europe, but that is inevitable. The mood has changed a great deal since the day in 1940 when the then British Prime Minister, Winston Churchill, flew to France to propose a political union of the two states. (The offer, hastily conceived, was turned down.) Curiously, life is altogether more serious now that it does not seem that it is about to be snuffed out. But as in any polite society, it is important that President Mitterrand's visitors at Fontainebleau should not make fun of him for his enthusiasm or, worse, suppose that he was not serious (which in some sense may nevertheless be true). The British among Europeans are notoriously given to the practice of a maddening pragmatism, and are at risk of forgetting that if

President Mitterrand were only half serious, he may nevertheless have hit on a way that will appeal to others of circumventing present impediments in Europe. The best hope at Fontainebleau is that both the British and their detractors will find some way of bridging the gap between short and long-term interests. The best field in which to look for a bridging formula is that for wringing from Europe's academic and research institutions the promise of which they are richly capable. □

One treaty to sign

One or other of the nuclear powers should urge that the threshold test-ban treaty be ratified.

ON the face of things, there seems no prospect of an early improvement in relations between the Soviet Union and the West. Even the proposal that the president of the US National Academy of Sciences, Dr Frank Press, should go to Moscow to try out some unspecified plans for further collaboration has been put off by accumulating doubts about the welfare of the Sakharovs (see p.485). This week's economic summit in London, which has brought together many of those who might have been able to influence events, was constructed around a different agenda (and a pretty empty one at that). Next will come the Northern Hemisphere's vacations and then the presidential election campaign in the United States, into which President Reagan will plunge without the once-hoped-for deal with the Soviet Union on some form of arms control. On present form, it will be a full year before anybody who matters will be in a frame of mind to take constructive steps.

Here, then, is a simple recipe for action now, not sometime in the future: dust off the Threshold Test-Ban Treaty and see that it is ratified by the United States and the Soviet Union. The treaty has been signed for the past decade, since when both the major nuclear powers have publicly insisted that they have kept to the letter of the treaty, which forbids the testing of weapons yielding more than the equivalent of 150,000 tons of conventional explosive. (Because of the earlier partial test-ban treaty, such tests must of course be underground.) While there have been complaints in the past few years, especially from the United States, that the treaty has been violated, the seismic evidence on this point is inconclusive or, more accurately, is consistent with the view that both the major powers have kept their unratified bargain with each other and the rest of us.

So why bother to ratify the treaty, in the process running the risk of a refusal by the US Congress? There are several practical reasons, not the least of which is that the full text of the treaty includes provisions for the exchange of seismic data about the neighbourhood of known testing sites that make the remote monitoring of other people's nuclear explosions less ambiguous, perhaps much more precise. It seems strange that neither of the major powers has sought to reap these benefits — or to shame the other by provoking a public declaration of unwillingness to ratify an otherwise agreed document. That, however, is only one of a long list of reasons why reasonable powers should legally endorse a reasonable document.

The threshold treaty may be a pale shadow of, say, an agreement on European missiles or a further instalment of the strategic arms agreements, but it has been negotiated and the others have not. And while it may not obviously be the "substantial" step towards strategic arms control required of the nuclear powers by the non-proliferation treaty, it is much more than the nothing that, on present form, the nuclear powers will have to report to next year's review conference of that treaty. Finally, the threshold treaty offers obvious scope for extension — reductions of the threshold, remote but on-site inspection — that could help to provide later and more ambitious agreements. It should not have escaped the US Administration's notice that a ratified agreement would also give the president something to boast about in November. The trouble is that there is very little time to act, with the party conventions in the United States only weeks away. That is another argument for moving quickly. □

Shoestring research

The problems of British research are epitomized by this week's closure of a laboratory.

WHILE nobody is conspicuously to blame that one of the best-known British laboratories in the neurosciences is to be closed (see p.486), the affair should give pause to those responsible for the management of research in countries such as Britiain, but in Britain in particular. And while the Medical Research Council (MRC) is on this occasion lucklessly responsible, there is every reason to expect the laboratories under the wings of other organizations will similarly find themselves in trouble when the time comes to replace a director who has decided (for good personal reasons) to quit. What has happened is a depressing symptom of what is wrong with the condition of British science.

The MRC Neurochemical Pharmacology Unit has its origins in the council's general policy, going back to before the Second World War, that research can be powerfully stimulated in important fields by providing a scientist of distinction with a good laboratory and a team of close colleagues with whom he can expect to work for decades to come. Of necessity, these units are small and also impermanent. If the good scientist should die, retire or simply quit, the case for the perpetuation of the unit may disappear at the same time. Ironically, the Cambridge unit partly owes its existence to the disappointment caused twenty years ago by the departure for the United States of the then newly appointed head of a similar initiative at the University of Birmingham. On this occasion, the council seems sensibly to have decided that the work of its unit at Cambridge was important enough to justify the search for a successor to the departed director. The outcome, by all accounts, has been disappointing. Part of the trouble seems to have been the search committee's confusion about its objectives. Should it seek a more or less classical pharmacologist? An up-to-date neuroanatomist? Or a molecular biologist? The difficulties seem to have been aggravated by some candidates' agony in making up their minds and by other people's choosiness about candidates with whom they would or would not work. In the event, the mere passage of time seems to have put paid to the hope that the unit could be kept alive.

Recriminations will now abound, but are inappropriate. To spend more than two years looking for a suitable person and then to fail to find one may betoken indecisiveness but is more vividly a proof that Britain, even Cambridge, is no longer a kind of mecca for professional people from elsewhere. Those who think otherwise might reflect on the recent fall in the value of the pound sterling (at or below $1.40), but there are more serious difficulties. A unit of a dozen or so people may be able to make an important mark in some chosen field if it has been welded together by some central instigator, but is otherwise probably too small to be able to hold its own against the competition. An attempt to create in Britain a unit on a scale that would be capable of holding its own in the exciting but competitive game of telling which genes do what in neurones would be doomed to failure now that speed in working out the nucleotide sequence of a gene has apparently became crucial. The particular lesson for MRC is a further narrowing of the scope for setting up research units on the now-familiar pattern. And just as the Science and Engineering Research Council is being forced by circumstances to reconsider its role in the support of high-energy physics, so MRC may soon find that some fields of once-cheap research are beyond its means.

The other lesson, for British research managers of all kinds, is the importance of flexibility. Historically, MRC's units have been successful because it has been possible to found them quickly in emerging fields of interest, and without uprooting people in the process. There will no doubt continue to be scope for entrepreneurship of that kind. But if competitiveness is important, there can be no preordained pattern or scale for the operation of such units. Those which succeed must be encouraged to grow, even in directions not foreseen. Others, less successful than the Cambridge laboratory, must be allowed to lapse. Doing nothing is the worst policy. □

US genetic regulations

Bacterial field trial to go ahead

Washington

TWO weeks after a federal judge halted Dr Steven Lindow's planned field trial of recombinant ice-nucleating bacteria (see *Nature* 24 May, p.296), the Recombinant DNA Advisory Committee (RAC) of the US National Institutes of Health (NIH) approved a virtually identical proposal from Advanced Genetic Sciences Inc. (AGS), at its meeting on 1 June.

The Lindow experiment was blocked by Judge John Sirica in response to a motion for a preliminary injunction filed by anti-genetic-engineering activist Jeremy Rifkin. The judge's ruling also barred RAC from approving any other experiments involving release of recombinant organisms to the environment until a full hearing can be held on Rifkin's claim that RAC must prepare an environmental impact statement before proceeding in this area. According to NIH officials, preparation of such a statement would require at least a year. (On 25 May, the Court of Appeals in Washington upheld Sirica's preliminary injunction against the Lindow experiment.)

Sirica's decision specifically exempted commercial proposals, however, which are not in any case legally bound by RAC decisions, although companies have been submitting proposals to RAC for review in keeping with the standard practice of voluntary compliance with the RAC guidelines.

At last Friday's meeting, Rifkin asked the committee not to establish a "double standard" that would give private companies an unfair advantage over federally-supported research, which is legally bound by RAC decisions. Robert Mitchell, chairman of the committee, responded that he had ruled that RAC would continue to consider commercial proposals, noting that they were specifically exempted from the court's decisions, which in any case applied only to NIH administration, not the RAC itself, which is legally an advisory body to NIH.

RAC took noticeably greater care this time to spell out its reasons for believing that the ice-nucleating experiments do not pose a risk to the environment. Dr Anne Vidaver, a plant pathologist from the University of Nebraska and a consultant on both the Lindow and the AGS proposals, noted that the modified bacteria to be used in the trials — so-called INA–, for ice-nucleating activity minus — already exists in nature along with the INA+. The INA– are lacking a gene responsible for the ice-nucleating protein, a protein believed to mediate frost damage to plants at temperatures a few degrees below freezing. Vidaver said that in natural populations of the bacteria (a variety of *Pseudomonas syringae*) as much as 80 per cent may be INA–. She said that apparently the only reason for using recombinant DNA techniques for producing the INA–, rather than simply culturing naturally-occurring ones, is that natural organisms are not patentable.

Vidaver recommended several modifications in the AGS proposal: an increase in the frequency of monitoring of the field plot, the withholding of approval of continuing experiments involving other, unspecified varieties of INA– *P. syringae* and a limit of the tests initially to one growing season, rather than the three requested. If preliminary data show no problems, she said, approval for additional tests should be given at that time.

At its 1 June meeting, the committee also approved a proposal from Cetus Madison for testing an undisclosed plant variety (the decision was made at a closed session of the committee) and adopted a set of "points to consider" for researchers preparing future proposals for plant field trials. The committee noted that past submissions have often been complete, necessitating their return for additional information. The list of points calls for proposals to include details on specific cultivars, vectors (including documentation that the vectors will not acquire pathogenic potential), data from greenhouse studies and monitoring procedures.

The committee also approved lowering containment of a previously approved toxin-cloning experiment from P4 to P3, or to P2 if the clones prove to be of low toxicity. The proposal, submitted by Dr Alison O'Brien of the Uniformed Services University of the Health Sciences, a part of the Department of Defense, involves cloning a shigella-like toxin in *E. coli*. The aim is the development of a vaccine against dysentery.

Stephen Budiansky

Telecommunications

Luxembourg goes for cable

TINY Luxembourg has thrown a spanner into the comfortable world of European telecommunications. In particular, Luxembourg has deeply offended the government of France by announcing that it plans to license a commercial consortium to launch communications satellites capable of delivering television broadcasts to cable networks throughout Europe.

Among European governments, France is the most deeply offended because France has been relying on Radio-Tele-Luxembourg, the commercial radio and television company based in Luxembourg, to pay towards the cost of the direct broadcasting satellites which France plans to launch in the next few years. Although Luxembourg, like other members of the International Telecommunications Union, has been allotted space in geosynchronous orbit for satellites capable of broadcasting signals powerful enough to reach individual receivers, the Grand Duchy appears for the present not to plan to take up this option.

Instead, the government of Luxembourg is planning to license the Société Luxembourgeoise des Satellites (SLS) to launch less powerful telecommunications satellites. The company, whose chairman is Mr Corneille Bruck, the president of the state savings bank of Luxembourg, will eventually operate under the name of Coronet.

For Luxembourg, which relies on Radio-Tele-Luxembourg for a substantial fraction of its revenue, the development is something of a surprise. While some observers consider that the move spells an end to the cosy relationship, some say identity, between the government and Radio-Tele-Luxembourg, others consider that the government may simply be insuring against the chance that direct broadcasting satellites will be unprofitable.

French uneasiness about the new plan seems to centre on the threat it poses to the commercial viability of the French direct broadcasting satellites. There is also some uneasiness within the European Telecommunications Satellite Organization (EUTELSAT), the European equivalent of INTELSAT. A meeting of the organization, on which national telecommunications authorities, mostly nationalized, are represented, held in Paris towards the end of May, shrank from condemning Luxembourg for a divisive move and instead urged on all its members the need not to take part in competitive international telecommunications services. Luxembourg insists that the plan for Coronet, aimed at delivering broadcast signals only to the operators of cable networks, escapes that criticism and so should be free from constraint.

However this may be, the row between Luxembourg and its larger neighbours has vividly drawn attention to three points. First, Luxembourg is determined to remain a principal exporter of broadcast signals even in the new technological age. Second, national susceptibilities as to who should be free to broadcast to whom are as fierce as they have ever been. Third, the commercial future of direct broadcasting satellites, capable of reaching individual homes, seems to be clouded by Coronet's demonstration that low-powered satellites coupled to cable systems can be built quickly and cheaply. □

Soviet jurisprudence

Nature letter heading for trial

New York

A LETTER from ten Moscow scientists published on *Nature*'s correspondence pages in December 1981 (**294**, 688; 1981) may be cited as nominal defendant in a prosecution under Article 190/1 of the Russian Republic's criminal code, according to a recent Western visitor to Moscow. During the past two years, the signatories remaining in the Soviet Union (two have been allowed to emigrate) have on several occasions been called into the procurator's offices as "witnesses" against the letter. Formally the case is still pending — but the visitor met separately two of the signatories and members of their families all of whom said that pressure was mounting and they feared that formal proceedings might start soon. The plight of one signatory, Dr I.S. Irlin, is referred to in Correspondence (p.490).

The letter drew attention to the signatories' "hard and humiliating" plight since they had filed applications to emigrate to Israel. "Almost all of us have lost our jobs", they wrote, "and we are denied any possibility of continuing with our scientific research. The doors of scientific institutions, laboratories, seminars, conferences and symposia, editorial offices and publishing houses are shut in our faces. We are being deprived of our scientific degrees . . . We have in fact been thrown out of society, but at the same time are not allowed to leave the Soviet Union."



One of several lines adopted by the Soviet interrogators was the legal fiction that the letter might be a forgery. They accordingly demanded that the signatories give evidence on this point. So far, it appears that all have refused either to confirm or deny the authenticity of their signatures.

The rationale behind the odd piece of jurisprudence of accusing the letter itself and not the signatories is simple — in Soviet law, accused persons have the right to stay mute, but witnesses have no such privileges. Already on at least one occasion, the ploy of "prosecuting" a document has been used. Dr Viktor Brailovskii, the mathematician who for many years hosted the unofficial Sunday seminars for refusniks and who has just completed a term of Siberian exile under Article 190/1, was originally called as a witness against the *samizdat* journal *Jews in the USSR*, which he edited. During the case new charges were framed against him, and he ended up first in the dock and then in Siberia.

The same ploy may therefore be expected against one or more of the eight remaining signatories (L.A. Dikii, M.I. Freidlin, I.S. Irlin, A.L. Vasilevsky, M.I. Reitman, S.A. Katz, Ya V. Medvedkov and V.N. Soyfer). The prosecution is clearly keen to get its evidence. The wife of one of the signatories has reported that she has been repeatedly approached by a "friendly" KGB man who urged her that it would be "best" for her husband to testify.

Events seem to follow closely on the trial of Brailovskii. The letter in *Nature* and the accompanying leading article are well known in the Soviet Union. In the circumstances, it seems strange that news of a possible case touching on editorial policy should reach the editor only through the unofficial channel of a returning academic visitor. **Vera Rich**

Soviet Jewry

Refusnik flow slows to trickle

Jerusalem

DR Yoram Dinstein, rector of Tel Aviv University, last week announced a new endowment fund to establish university posts for Jewish scientists emigrating from the Soviet Union. The timing was somewhat inauspicious. Israeli universities are so short of funds that six months ago it was feared that the entire higher education system might have to close down altogether. Even now, no vacant posts can be filled, and all non-tenured academics are under threat of redundancy. Positive discrimination in favour of new arrivals from the Soviet Union could be a major cause of friction.

The endowment fund has been set up by Mr Fabian Kolker of Baltimore, Maryland, a governor of the university, as part of his overall strategy to halt the problem of the *nashrim* — Soviet Jews who emigrate on a visa for Israel but who then settle elsewhere, usually in the United States. But the rules of the fund allow the annual interest to be used to help Israeli scientists seeking academic posts should there be no suitable new immigrants from the Soviet Union.

Although Jewish emigration from the Soviet Union is now down to around 50 a month (from a regular 3,500 a month in the early 1970s and a freak 4,500 a month in 1979), the fund has already been used to create a post for one recent arrival, Dr Grigorii Freiman, a mathematician. But under present circumstances it may be some time before another Soviet candidate arrives.

The clampdown on emigration has focused attention once again on the estimated 300 Soviet Jewish scientists who have already filed visa applications but who have been refused permission to leave. Deprived of their scientific posts, "overqualified" and therefore unemployable in other jobs, refusniks are now liable under new Soviet legislation to arrest for "parasitism" if they are without visible means of support for more than two months.

Since 1964, refusniks have sought to keep up their scientific work through unofficial seminars and even international conferences, the first of which was totally frustrated — the Soviet would-be participants were temporarily deported from Moscow and invited foreign participants denied visas. Last week, an anniversary conference was held at Tel Aviv University (also on the initiative of Mr Kolker), with scientific papers presented by, among others, Professor B. Pippard, Professor E. Segre, Professor P. Kessler, Professor H. Markovich and the Israeli Minister of Science, Professor Yuval Ne'eman. Former refusniks taking part included Professor Benjamin Levich (a Corresponding Member of the Soviet Academy of Sciences), Dr Eytan Finkelstein, Professor Alexander Voronel who founded the refusnik seminars and Professor Mark Nobel, who succeeded him as convener.

A number of papers by present refusniks were read *in absentia*. The principal aim of such gatherings (including previous conferences in the United States and Stockholm) is to present the refusniks' work in its scientific content and to demonstrate that the seminars (which still continue in Moscow and elsewhere, albeit somewhat less regularly than previously) can generate meaningful work.

The question arises, however, whether such special meetings are the only or the best way of presenting the refusniks' work. Invitations to international conferences abroad are, of course, only symbolic gestures, while conference programmes are now too crowded to permit the custom of the 1960s of keeping blank the time-slot allotted to a Soviet participant who fails to arrive. Even papers sent to be read *in absentia* will usually be crowded out.

One approach not apparently tried so far is to encourage refusniks to submit contributions for poster sessions. Many of the papers at the Tel Aviv conference were, in fact, of poster length, having been dictated by telephone.

With a scientific colleague abroad as sponsor, such participation could prove a valuable way of keeping the refusniks in the world scientific community. **Vera Rich**

Sakharov

US academy cancels visit

Washington

MOUNTING anxieties about the healthof Dr Andrei Sakharov and his wife Yelena Bonner have forced the US National Academy of Sciences to postpone a planned visit to the Soviet Union next week. Dr Frank Press, president of the National Academy of Sciences, announced last month that he planned to deliver new proposals for resuming formal research links with the Soviet Academy of Sciences. The US academy decided to suspend the links in 1980 as a protest against a Soviet Government decision to condemn Dr Sakharov to internal exile in the town of Gor'kii, some 250 miles east of Moscow.

An academy spokesman said the visit was now in abeyance but the condition of the Sakharovs was being monitored daily. In a telegram sent jointly with the Royal Society of London, the Academie des Sciences de L'Institut de France and the Royal Swedish Academy of Sciences, the US academy at the end of May called on its Soviet counterpart to "act as effectively as possible to help Academician Sakharov and his wife in getting the health care they require and request".

The sudden crisis over the Sakharovs could hardly have come at a worse time for the academy, which had already come under criticism from some quarters for its decision to seek a resumption of ties with the Soviet academy. In an interview on 23 May, for example, Dr George Keyworth, President Reagan's science adviser, said he was "rather surprised" by the decision.

Dr Press has consistently refused to provide details of the proposals he would be taking to Moscow, but apparently hopes to revive the relationships that existed 20 years ago, when distinguished scientists from both countries took part in exchange programmes. Since the US and Soviet academies established a formal exchange programme 25 years ago, 500 scientists have travelled in each direction, but the volume of exchanges dwindled after the US academy declared a moratorium over the Sakharov affair. During 1983, there were a few individual exchanges; 26 Americans visited the Soviet Union for a total of 38 months while 13 Soviet scientists spent a total of 31 months in the United States.

Reaching a new agreement with the Soviet Union may prove difficult even if the political controversy surrounding the Sakharovs abates. Although the US academy is keen to resume links, it wants to change the way Soviet scientists are selected for visits to the United States. US scientists have often complained that under previous exchange agreements, too many junior scientists were being selected by the Soviet Union for visits to the United States.

Peter David

Star wars

Sceptical report from OTA

Washington

THE Reagan Administration has responded with unusual ferocity to a report by Congress's Office of Technology Assessment (OTA) that casts doubt on the ability of the United States to build a space-based "star wars" defence against nuclear attack. Lieutenant General James Abrahamson, former shuttle director and newly-appointed head of the star wars initiative, has told Congress that the report contains "technical errors, unsubstantiated assumptions and conclusions that are inconsistent with the body of the report".

The OTA study, published at the end of April, is the only detailed technical analysis of the star wars idea yet to be published by a neutral government body with full access to classified research data. Its author, Dr Ashton Carter of the Massachusetts Institute of Technology, concluded that the prospect of being able to develop a "near-perfect" defence against nuclear missiles was so remote that it should not form the basis of public policy.

Although they cleared the report for publication, a number of high-ranking defence officials have complained privately that it contained sensitive details of progress in research that should not have been disclosed. A Republican congressman, Henry Hyde of Illinois, has threatened to launch an enquiry into OTA's "alleged security breaches" and to propose legislation making semi-autonomous bodies like OTA "more accountable" in handling sensitive information.

OTA, however, dismisses the allegations of a possible breach of security. Dr Carter, a former DOD analyst specializing in anti-ballistic missile systems, maintains that the report discloses next to nothing about the current state of US research on any of the sensitive technologies. And in a point-by-point rebuttal of General Abrahamson's technical criticisms, OTA insisted last week that its report was technically correct.

Because they are hamstrung by secrecy requirements, neither side in the debate has been able to substantiate its technical criticisms and rebuttals. Much of General Abrahamson's report, for example, consists of simple assertions that OTA based its conclusions on outdated information and was therefore too pessimistic. In response, OTA has been able to say only that it is familiar with all the latest work and that its conclusions are fair.

One of the main technical quarrels is about the use of new fast-burn missiles which, OTA believes, might thwart a star wars defence because their rockets would burn out while they were still in the Earth's atmosphere. The administration's initiative stresses the importance of intercepting missiles during their boost phase, while they can be easily detected by their exhaust plume and before they deploy their multiple warheads. Fast-burn boosters would be vulnerable for a much shorter time and the atmosphere would help to shield them from several futuristic weapons such as neutral particle beams and X-rays.

General Abrahamson, however, rejects OTA's view that the deployment of such boosters by the Soviet Union could prove a "potent, even decisive" countermeasure against directed energy weapons. It would, he says, take many years and a lot of money for the Soviet Union to deploy a large fraction of its arsenal on such boosters. Even then, their use would reduce the payload and accuracy of their missiles by between 70 and 90 per cent. OTA counters that "nothing like" a reduction of that magnitude would be necessary; it believes fast-burn boosters could be designed to deploy heavy payloads accurately enough to destroy US missile silos, and certainly cities.

OTA and DOD also disagree about how well lasers, X-rays and particle beams could perform as star weapons. General Abrahamson says OTA underestimates the effective range of chemical lasers and disregards promising (but secret) progress in the range of X-ray lasers and neutral particle beams and their ability to penetrate the atmosphere. According to the general, new advances enable a "modest" constellation of beam weapons to "negate most ballistic missile threats". And he dismisses OTA's argument that satellite battle stations and the other space-based paraphernalia of a star wars system would be extremely vulnerable to attack; he says a number of effective means have been developed to ensure that satellites could survive an attack.

General Abrahamson's response to OTA may well have been unusually sharp because it was published before it could be reviewed by the national laboratories most closely involved with star wars research. In subsequent reviews, the general claims, experts at the laboratories found the report full of technical flaws. Even this claim, however, is in dispute; Dr Carter says many scientists at the laboratories support OTA's conclusions.

Whatever its merits or defects, the report has made its mark in Congress. The House of Representatives' subcommittee on international security and scientific affairs cited it in a critical report last month on the administration's space arms control policy. The report said Congress had received no "conclusive" evidence that a perfect or near-perfect defence is technically feasible. It added: "As has been the practice between the superpowers in the nuclear age, the practice of each side developing a countermeasure to the other side's systems could continue unabated under the US and Soviet strategic defence programmes."

Peter David

UK medical research

Neurochemical unit threatened

ONLY a few months after advertising its intention to spend more on research in the neurosciences, the British Medical Research Council (MRC) is taking steps to close one of its better-known units in the field, the MRC Neurochemical Pharmacology Unit at Cambridge. The decision, made known to the staff last week, comes two and a half years after the then director of the unit, Dr L.L. Iversen, announced his appointment as director of a new research laboratory established by Merck, Sharp and Dohme at Hoddesdon in Hertfordshire.

MRC's decision, described as a "decision in principle", will be controversial. The unit has in the past twenty years acquired a reputation for the development and understanding of neuroleptic drugs, chiefly by the refined application of classical pharmacological techniques. More recently, the unit has taken an interest in the histological examination of post-mortem brains, especially from people with schizophrenia.

The unit, one of several maintained by MRC, usually in close association with a university, consists of a score of people of whom perhaps a dozen are established scientists. In its heyday, the unit also played host to large numbers of postdoctoral fellows from elsewhere. The budget for the current year is £375,000.

There is some confusion about the reasons given for closing the unit, said last week to have been made necessary by the difficulty of recruiting a suitable successor to Iversen, at least within the limits of what the council had been prepared to pay for the job. The council said earlier this week that the post had been widely advertised and that the salary offered had been the equivalent of that of a university professor.

On the other hand, Dr Leslie Iversen, the previous director of the Cambridge unit, said that he was "furious" at MRC's decision in principle that the unit should be closed. He said that he had only recently been consulted about at least one eminently suitable candidate who had been interested in the job. He said he planned to write in protest to the secretary of the council, Sir James Gowans, saying that the appointment of his successor had been mishandled and that he doubted whether the council would be able to economize by closing the unit, given that many of the members of the unit were tenured members of the council's staff and that the space vacated by the dispersal of the unit would quickly be occupied by others.

Another factor in the council's decision seems, however, to have been the British Government's refusal of its request for extra funds to sustain an expansion of research in the neurosciences. The council is understood to consider that with recent developments at Imperial College, London, Oxford, Edinburgh and Newcastle, it has done as much as could be expected to strengthen the pattern of British research in this field.

A further difficulty seems to have been the changing composition and objectives of the search committee, which at various times seems to have been anxious to confirm the previous pattern of research in the Cambridge unit and at others to wish to branch out in new directions. The search seems to have been in abeyance for a full six months early in the proceedings, when one candidate to whom the job was offered found it hard to make up his mind.

On other occasions, the committee seems inexplicably to have tried to tempt distinguished researchers from the United States with inadequate rewards. The salary offered was formally stated this week to be the equivalent of that of a university professor although it is known that at least to one candidate from overseas figures in the neighbourhood of £30,000 a year were mentioned and not scorned.

The immediate future of the rump of the Cambridge unit is far from clear. The next step is that a management committee of council scientists will visit Cambridge to hear what plans the remaining scientists may have and eventually to make recommendations to the council.

Dr P.C. Emson said from Cambridge earlier this week that interest among potential postdoctoral fellows in work at the Cambridge laboratory had sharply declined in the past six months. He also said that MRC had only a few months ago given the unit the right to fill a research studentship from the beginning of the coming academic year but had then "taken it away again last week".

Even at this late stage, it seems that at least part of the rump could be reprieved. One possibility that has been suggested is that the remaining members of the unit might be able successfully to persuade MRC to support them with a five-year project grant. Most observers, however, consider that those concerned will quickly be tempted to take posts elsewhere.

John Maddox

High-energy physics

UK debate goes public

THE Kendrew committee set up to review British participation in Europe's high-energy particle physics has now asked members of the research community for their views. The group particularly wants opinions about the relevance and standing of the current UK effort, technical and educational spin-off and of any problems of the European Organization for Nuclear Research (CERN).

Others besides particle physicists may feel moved to respond: the review group will also be pleased to hear about other new areas of science, or grossly underfunded existing areas, that would benefit from a significant increase in support.

The review, under the chairmanship of Sir John Kendrew, was set in motion earlier this year at the instigation of the Advisory Board for the Research Councils, which is studying the question jointly with the Science and Engineering Research Council (SERC). The council expects to spend £52.6 million on high-energy particle physics in 1984–85; £35.6 million of this is the UK subscription to CERN, while most of the remainder relates indirectly to work at CERN. Strong competing claims on the science budget led to questions about whether the high cost — almost one fifth of SERC's expenditure — was justified.

The committee expects to complete its onerous task within a year, and is planning a hectic programme of activity. Apart from the obligatory visit to CERN in Geneva, it will visit other countries to see how their ideas compare with those in Britain. Germany and the United States are provisionally scheduled for visits, and the committee will also visit British laboratories that work in particle physics.

Legal advice has been sought on the question of Britain's contractual obligations to CERN. It seems to be recognized that Britain could legally withdraw from CERN with one or two years' notice, although the political backlash would be severe. One view is that Britain is morally committed to CERN at least until the completion of LEP 1, the electron-positron collider now under construction and scheduled to start operations in 1987–88.

If, as seems possible, this view is accepted by a majority of the committee, the issues will centre on what should come after LEP. One possibility the committee hopes to look at is that of greater collaboration with the United States — although there are doubts about whether US scientists would be happy to forgo a machine based in the United States for one in Europe.

Speaking at last week's meeting of the American Association for the Advancement of Science, CERN's director general Dr Herwig Schopper suggested that the LEP tunnel could form the basis of a future proton machine with capabilities similar to those of the planned superconducting Super Collider (SSC) but at a much lower cost. The continuing uncertainty over US commitment to the SSC may make it hard for the Kendrew committee to come to any firm conclusions.

Tim Beardsley

Submissions to the review group should be sent to: Dr Keith Root, Room 5/37, Department of Education and Science, Elizabeth House, York Road, London SE1 7PH.

US research grants

New pork-barrel row breaks

Washington

A YEAR of tireless campaigning by university leaders in the United States has failed to keep "pork barrel" politics out of the 1985 science budget. Georgetown University in Washington DC is asking Congress to direct the Department of Defense (DOD) to support a $220 million energy demonstration project at the university without going through the usual peer review procedures.

The timing of Georgetown's initiative has horrified the scientific community in Washington, which has spent much of the year trying to persuade Congress that research funds should be distributed according to scientific merit determined by peer review. The campaign began last year when two universities, Catholic University in Washington DC and Columbia University in New York, employed a Washington-based lobbying firm to talk Congress into earmarking last-minute funds for scientific facilities at their institutions, although there had been no peer review in either case.

Those developments provoked a stream of resolutions and statements by scientific and university bodies stressing the importance of peer review. Since then, virtually every university spokeman testifying before committees on Capitol Hill has included a statement explaining that wise scientific investment requires that proposals should be assessed on their merits.

Last month, nevertheless, Georgetown's lobbyist, the Reverend Byron Collins, appeared before the House appropriations subcommittee on defence to request $9 million in 1985 to start work on the university's unsolicited proposal for a coal-gasification and fuel-cell cogeneration programme. The committee's first response has been favourable; last year, it agreed to a Georgetown request to convert $820,000 of army research funds to planning the project.

Two higher education associations, the Association of American Universities and the National Association of State Universities and Land Grant Colleges, are spearheading a drive to block the Georgetown project. A staff paper circulating within the associations accuses Georgetown of consistent attempts to use its political clout to win research funds. "Over many years", the document says, "the university has taken great advantage of its location to cultivate key members of the Congress and to gain their support for legislation granting the university special treatment . . . Georgetown initiatives are almost uniformly designed to circumvent the established competitive merit-based system for the award of university program funding".

Peter David

Animal legislation

California pounds stay open

Los Angeles

CALIFORNIAN researchers won a major victory last week in their continuing battle with antivivisectionists. A proposed measure to prohibit the use of impounded stray animals in experiments was defeated by 9 votes to 4 in a state assembly committee after intensive lobbying by a coalition of Californian universities and biomedical organizations. The bill, sponsored by state Senator David Roberti of Los Angeles, passed the California senate last year by a healthy margin. It would have prevented animal pounds in California from selling or giving away animals for research and would also have prohibited the transfer of research animals from one state to another.

The bill's defeat is now being interpreted as a major coup for medical researchers throughout the United States. Animal rights groups believe that if such a law had been passed in California, few states would have failed to follow. Nine have already enacted laws that forbid impounded animals from going to research laboratories but researchers in eight of these are allowed to import animals from other states. Only Massachusetts, which passed its animal protection bill several months ago, intends to forbid importation, and as a consequence it is estimated that the costs of experiments using dogs and cats will increase tenfold.

In their successful lobby against the bill, California researchers argued that it would have cost $12 million to establish breeding facilities for dogs and cats and some $8.5 million a year to run them. They noted that about 15.5 million unclaimed animals are killed in California's pounds each year, and that they take only 3 per cent of such animals to laboratories for experiments.

Senator Roberti seems to accept that the bill is dead. He says that he will recommend to humane groups that "the avenue available to them to change the hearts and minds of the legislature is to appeal directly to the people". Senator Roberti said he was not against animal research but, rather, against experiments on pets. But an alternative bill that would prevent pounds from turning "proven" pets over to researchers is languishing in the state senate because few people think it is enforceable.

Meanwhile, animal protection groups in California are livid. "The power of the University of California is overwhelming", said Gretchen Wyler of the Fund for Animals. "They are in control of this state." The next step, she said, will be to go to Washington DC to try to enact federal legislation.

The lobbying success of university and biomedical groups in California was extraordinary. Every major California newspaper supported them, while heart transplant recipients and blind diabetics pleaded before elected officials for continued animal research. Even so, the agony of the decision was clear. Committee chairman Richard Alatorre, as he cast his vote, said that a recent television programme on animal research had been a clear indictment of the academic community and of its conduct of research; but, he said, a good friend who died of leukaemia had begged him to vote for continued research on animals.

Sandra Blakeslee

Raid on US baboon laboratory

A GROUP calling itself the Animal Liberation Front has broken into a laboratory at the University of Pennsylvania, vandalizing equipment worth thousands of dollars and stealing 20 videotapes of experiments on baboons.

The laboratory, directed by Dr Thomas Gennarelli, has conducted experiments with baboons designed to simulate traumatic head injuries to people as part of the university's head injury research programme supported by a $1 million grant from the National Institute of Neurological and Communicative Disorders and Stroke.

A spokesman for the burglars, who have been identified only as "five members of the Animal Liberation Front", said that Gennarelli's laboratory was chosen for the break-in because his experiments are "notorious" and because of rumours that he had been filming them. Copies of selected tapes provided to local television stations by the burglars show the researchers making jokes about brain-damaged monkeys, and have provoked predictably hostile public reaction.

Dr Thomas Langfitt, principal investigator of the research programme, defended the as humane and vital for the improvement of the treatment for head injuries. The injuries are induced by accelerating the baboons' heads, without impact. Langfitt said the research has already revealed the mechanism by which damage occurs in such accidents; results have lead to mathematical and physical models of the axons and blood vessels in the brain during injury and to the design of improved crash helmets. Langfitt said that the baboons are tranquillized and lightly anaesthetized during the procedure. All the animals are eventually destroyed.

The Animal Liberation Front says it will turn over to the US Department of Agriculture evidence of violations of the Animal Welfare Act. The act requires the proper care and housing of animals, but leaves experimental design entirely up to the researcher.

Stephen Budiansky

Forestry

Taxing changes in prospect

POLICY proposals published this week by Britain's Forestry Commission include for the first time special provisions for the protection of Britain's ancient semi-natural woodland. In a consultative paper, *Broadleaves in Britain*, the commission suggests measures to minimize loss of all broadleaved woods and introduces guidelines agreed with the Nature Conservancy Council (NCC) for managing ancient semi-natural woodland. This change of heart by the commission, which previously failed to recognize this category at all, is seen as a major concession to conservation interests.

According to a survey (as yet incomplete) by NCC, losses of ancient semi-natural woodland over the past 40–80 years in different counties range from 10 per cent to over 60 per cent, with most counties so far surveyed showing losses of over 40 per cent. The commission, which advises private foresters and manages its own forests, now recognizes a good case for slowing the rate of future loss, and proposes new management guidelines that will automatically check felling proposals and grant applications against the NCC register of ancient woodland. For woods so categorized there will be "a presumption in favour of maintaining the species already present" and owners will be persuaded to adopt methods of working that are "least disruptive of the woodland habitat". Broadleaved woodland in general should be maintained at about its present extent by requiring replanting with broadleaves after felling and allowing permanent clearing "only exceptionally".

The Forestry Commission has been under intense pressure from conservation groups to change its policies in this area, and has consulted extensively with interested parties for more than two years. There was therefore some surprise this week that the new document is consultative and not a definitive policy statement. Comments can be made on the proposals even at this stage. The commission hopes nevertheless to offer its final advice to government before the end of the year, as proposed tax changes and higher grants for establishing broadleaved woodlands will need ministerial approval.

Most of the broadleaved woodland in Britain is in private hands. Timber Growers UK Ltd, which represents foresters' interests, welcomes the Forestry Commission's proposals as a big step in the right direction. But, in the view of the association's chief executive, Mr Ronnie Williams, the commission could have been much firmer. Private foresters are keenly aware of the need to improve financial provisions for owners of broadleaved woodland, who are at a disadvantage compared with owners of the faster-growing and more profitable conifer stands. The Forestry Commission now proposes to make it much easier for owners of mixed estates to claim tax relief on slow growing broadleaved plantations, a move welcomed by the timber growers. But, they say, the commission does not go far enough. Only by making broadleaved woodland management less of a financial burden in the hundred years or more from plantation to financial returns will the pressure to destroy ancient woodland be removed. It is also pointed out that the commission has hardly been providing a good example by its own planting policies: the vast majority of recent Forestry Commission plantations are of conifers.

The Forestry Commission makes much of the fact that the total area of broadleaves in Britain is now 15 per cent greater than in the 1940s, and its proposals could be briefly summed up by saying they aim to maintain the *status quo*. Private foresters, however, argue that a million acres of woodland were lost in two world wars and want a return to the levels of the turn of the century. But at present there are severe economic obstacles to broadleaved production, mainly the high costs of establishments, the low productivity and long rotations.

The Forestry Commission is currently reconsidering much of its research activity, and that on productivity of broadleaved species is one important area of discussion. The new *Broadleaves in Britain* document gives some indications of where it might be heading. The review group that produced the document considers it is "doubtful" whether improvements in growth rate can be achieved through the use of fertilizers, and in the long run the improvements will come about through genetics, most probably using vegetative propagation techniques and clonal testing. There is also, according to the group, a need for more information on total biomass production and how this is affected by spacing and harvesting regimes, as well as the properties of species mixtures and newly introduced species.

Tim Beardsley

European biotechnology

Optimism abated as summit looms

Brussels

OFFICIALS of the European Commission fear that the Community's proposed biotechnology programme is falling foul of the present crisis in the Community's affairs. One sign of this is that the powerful agricultural lobby is mobilizing to block certain reforms that would have helped the growth of a strong biotechnology industry.

In spite of the generally favourable reception of the proposed programme by both research and industry ministers in November and December last year, that conjunction of omens seems now to be dissipating. "The alliance is coming unstuck", complained one official, citing the objections now raised by the sugar beet industry to a new pricing regime for the industrial use of sugar. "The sugar beet industry thinks that the biotechnology programme is the thin end of a wedge" leading to lower prices which, the official guesses, is the reason why agricultural ministers are now dragging their feet.

The Commission, meanwhile, is trimming its optimism about biotechnology to the changing wind. Last year, the Commission asked for a five-year programme costing 200 million European Currency Units (ECU) (about £118 million) but in its revised proposal put forward on 26 April, the Commission asked for only 88 million ECU covering only two of six of the original proposals — research and training and "concertation" with other biotechnology programmes.

At this level, the programme would represent a little more than a million dollars per country per year, an order of magnitude less than the Esprit information technology programme. The Netherlands and Denmark are unhappy about the size of even this proposal, while the United Kingdom and West Germany want the programme cut back drastically. "The council seems intent on reducing the whole thing to ashes before they have to build the whole edifice again", said one frustrated scientist.

The cuts and delays are especially galling for officials still smarting from the criticism earlier this year from the US Office of Technology Assessment that Europeans are not aggressive in commercializing biotechnology and pose little threat to the US industries.

At the twice postponed meeting of science ministers, now fixed for 29 June, there is even a danger that discussion of biotechnology will be swamped by two other items of high importance — telecommunications and the fusion programme. A spokesman for Research Commissioner Etienne Davignon insists that biotechnology retains its high priority, but British officials complain that they have yet to see where it fits into the Commission's larger scheme of things.

A further imponderable is that, by the end of June, the next Community summit at Fontainebleau may or may not have resolved the Community's budgetary crisis. The Commission may decide to go for a decision in principle on biotechnology, letting the question of funds hang loose until later, but in any case the programme will have to await an opinion of the newly elected European Parliament, and so is not likely to see the light of day before next year. "But if things go badly at Fontainebleau", said one senior European official, "it could be a catastrophe for European research".

David Price

Federal laboratories

Packard calls for flexibility

New York

IMPORTANT changes in pay and conditions may be on the horizon for scientists employed in the United States' 700 federal laboratories, which include the National Insitutes of Health and laboratories run by the Departments of Energy, Defense and Agriculture. An interdepartmental committee headed by the White House science office has drafted legislation to free the laboratories from civil service pay regulations so that they can better compete with the universities and industry for talented scientists and engineers.

Addressing last week's meeting of the American Association for the Advancement of Science (AAAS), Mr David Packard, chairman of California's Hewlett-Packard Corporation, said the model legislation was proposed in a draft report to be submitted to President Reagan in July. But he predicted that the legislation would stir up considerable opposition from vested interests in the civil service.

Mr Packard, a personal friend of President Reagan, strongly criticized the civil service's inflexible procedures for pay and promotion in a review of the federal laboratories he conducted last year for the White House Science Council. The new legislation, he said, would create a separate personnel system for scientists and technicians, replace narrow pay bands with broad ones, waive the existing pay ceiling for senior employees and allow promotions to take account of merit as well as seniority.

The report going to President Reagan in July will claim there has already been impressive progress towards implementing other recommendations in last year's Packard report. In response to a call for clearer definitions of the missions of each of the national laboratories, for example, the Agricultural Research Service has for the first time drawn up long-term research plans for its institutions. And the Department of Energy has begun to make its laboratories produce five-year plans in which each institution must state its direction, role, expected changes and budgets.

But the director of the Oak Ridge National Laboratory, Dr Herman Postma, told AAAS that progress had been uneven. Both Congress and the Office of Management and Budget (OMB) continued to block a proposal to bring financial stability to the laboratories by setting rolling budgets for two or three years at a time. The Department of Energy, responding to Packard and earlier reports, had begun to give each laboratory an "exploratory budget" to be spent at its director's discretion, but the level was between half and one per cent of the laboratories' budgets instead of the 5 to 10 per cent recommended by Packard.

Peter David

Antarctic treaty

Minnows smell a rat

Tokyo

THE Antarctic Treaty Consultative Parties (ATCP) ended their secret deliberations in Tokyo on 31 May with the announcement that "good progress" had been made towards an agreement permitting the exploitation of Antarctic mineral resources. Representatives of nations that have no say in how Antarctica is to be carved up, as well as conservation groups that had come to Tokyo to monitor the meeting, took a less charitable view.

Of the sixteen members of the Antarctic Treaty, seven have territorial claims to huge areas of the continent which have been held in abeyance since the treaty was signed in 1959. Entry now is restricted to those who carry out a full programme of research in the Antarctic. Despite the very great cost of maintenance of bases and ice breakers capable of reaching them, both Brazil and India thought it worthwhile to join last year (see *Nature* **307**, 105; 1984).

Under discussion in Tokyo, at the Fifth Special Mineral Resources Meeting, was a plan put forward by New Zealand representative Chris Beeby. The meeting was surrounded by secrecy — despite repeated protests from the Netherlands, not even representatives of the fifteen nations that have signed the Antarctic Treaty but which do not carry out research there (the "Non-Consultative Group") were allowed to send observers. But copies of the original draft of the Beeby plan were circulated by conservation groups, and individual delegates were willing to discuss privately amendments made since the last meeting.

Essentially, the plan proposes that mineral exploitation be regulated by three types of institution. At the top is a central commission which will be the ultimate political body for the whole mineral regime, with membership restricted to the 16 ATCP members and to nations with definite plans for mineral exploitation. Next will come a scientific and advisory committee whose members will be appointed by the central commission, to provide "technical guidelines" including those for environmental protection. It will not, however, have any power to enforce these guidelines.

Real power will reside with "regulatory committees", one for each area of the Antarctic when a proposal is made for exploitation in that region. Each will contain representatives from the United States, the Soviet Union, four nations with territorial claims, the nation proposing mineral development and another nation nominated by it.

What this plan neatly achieves is an accommodation between those with territorial claims and nations that actually want to carry out mining operations in the Antarctic. Beeby speaks proudly of the great success that the Antarctic Treaty has achieved over the past twenty years in keeping international rivalries out of Antarctica. His plan is primarily concerned with maintaining this internationalist spirit while permitting mineral exploitation to take place.

But to the smaller nations the whole plan seems designed to keep power in the hands of the Antarctic Treaty members. Malaysia received overwhelming support from the last meeting of the non-aligned nations for a resolution calling for a UN debate on the Antarctic issue during 1984, a move that could end with a conference like that of the Law of the Sea which would call Antarctic resources, like ocean bed resources, the heritage of all mankind.

The non-aligned group accuses the treaty members of trying to tie up a minerals agreement to pre-empt such a move. Indeed, if environmental groups are right, preliminary investigations of Antarctic oil resources have already begun — despite a 1977 agreement to restrain exploration while "satisfactory progress" is being made towards a minerals agreement.

What disturbs the environmentalists more, however, is that there would be no publicly accessible central body with the power to ensure that the Antarctic environment is not damaged under the Beeby plan. To divide the Antarctic into regions, each under the control of an eight-nation group with an interest in exploitation, would, they claim, prevent any overall or consistent ecosystem management. And the Beeby plan contains no provision to regulate preliminary prospecting, even though a test drilling that went wrong could have appalling effects. There can be no guarantee that a nation will effectively discharge its obligation to supervise the mining operations of its nationals. Instead, the major conservation group concerned, the Antarctic and Southern Ocean coalition of 150 environmental organizations from 37 countries, is calling for an Antarctic Environmental Protection Agency to oversee all Antarctic operations. There is sympathy for this idea from some ATCP members, but not from the two superpowers.

That the Beeby plan has only been slightly modified since the last meeting of the Mineral Resources Group suggests that its form is close to being acceptable by the ATCP members and that the idea of an Environmental Protection Agency will stand little chance — although it is impossible to be sure, given the secrecy of the discussions. Discussions will continue in February 1985 with Brazil as the host country. There, for the first time, the non-consultative parties to the Antarctic Treaty will be allowed to send observers and it may be possible for more than a handful of nations to find out what is going to happen in Antarctica.

Alun Anderson

Pleas for would-be emigrés

SIR — We wish to draw your attention to the case of the respected Soviet cancer researcher, Dr Iosef Irlin, who has been involved in the fields of tumour virology and tumour immunology. His work has been of theoretical importance and in no way related to matters falling within any national security interests.

Dr Irlin applied four years ago for permission to emigrate with his family to Israel, to be reunited with other members of his family there, and he has reapplied repeatedly during the past four years. All his requests have been refused, without explanation. During this time, he has been without professional employment and his several applications for jobs have been rejected.

In an earlier letter, Dr Irlin states:

> It is now four years that I have been in refusal. As you must know well, being engaged in my profession, I have never been connected with any state secrets or security, dealing only with experimental oncovirology and immunology of experimental tumours — quite an open branch of science. In spite of this, my family and I were not allowed to emigrate to Israel and none of the officials, despite my numerous applications, took the trouble to give me any good ground for the refusal. By now, I repeat, for four years, I have been without a professional job and practically without hope. All my efforts to get any decent job have failed.

We appeal to scientists from all countries, including our Soviet colleagues, to use all their influence so that Dr Irlin, together with his family, is allowed to leave the Soviet Union to live and work in the country where he and his family wish to stay.

GEORGE KLEIN
JERZY EINHORN
Karolinska Hospital, Stockholm, Sweden
STUART A. AARONSON
National Cancer Institute, Bethesda, Maryland, USA
FRED RAPP
Pennsylvania State University, Hershey, Pennsylvania, USA
ANDRÉ LWOFF
Pasteur Institute, Paris, France
THOMAS GRAF
European Molecular Biology Laboratory, Heidelberg, FRG
DAVID BALTIMORE
Massachusetts Institute of Technology, Cambridge, Massachusetts, USA
ROGER WEIL
University of Geneva, Switzerland

SIR — I wish to support in the strongest possible way the appeal of Dr André Lwoff, and other Western biochemists and geneticists on behalf of Dr David Goldfarb whose exit visa for Israel has recently been withdrawn by the KGB (see *Nature* **308**, 679, 766 and **309**, 104; 1984).

I have known and admired Dr Goldfarb since he came to work in my microbial genetics unit at Hammersmith Hospital, London, some twenty years ago, and respected him as among the most honest, able and innovative Soviet geneticists of the post-Lysenko era. To those of us who have known Dr Goldfarb well, he has always reflected credit on the Soviet Union for his work on microbial genetics. I therefore found his harassment by the KGB when he applied for an exit visa in 1979 not only unjust but incomprehensible, especially in view of the assertion by the Soviet Academy of Sciences that he knew no state secrets. He also demonstrated his courage and patriotism at the siege of Stalingrad where he lost a leg. The latest withdrawal of his visa, again approved by the Academy of Sciences, on the eve of his departure for Israel savours of paranoid persecution and causes great distress to his many friends abroad in view of his advancing years and poor health. I therefore hope, in the interests of all those who support goodwill and international collaboration in science, that Goldfarb's case will shortly be resolved in his favour.

WILLIAM HAYES
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Skew physics

SIR — I was struck by the remarks on Fritz London in your recent article (*Nature* 10 May, p.109). Amongst other qualities, London was gifted with modes of apprehension of physical problems that were skew, or at any rate out of line with those of his contemporaries, with the consequence that some of his publications at first lacked the full impact their importance warranted. As some compensation, his originality enabled him to grasp more confidently skew developments presented to him.

I was fortunate to have personal experience of this trait in relation to my earlier letter (*Nature* **132**, 1002; 1933) on the change from "aromatic" to "metallic" electrons in organic compounds. This text was written with some unease because of its novelty, but on my walking back from posting it to your offices I was introduced to Fritz London by F.A. Lindemann, who was chatting with him at the entrance to the Old Clarendon Laboratory at Oxford. Rather tremulously, I showed them my letter. Lindemann read this politely, and said little, but Fritz London immediately grasped what it was trying to focus on about the unusual physical properties of aromatic molecules, such as their large diamagnetism, and reassured me. He himself later developed this theme more fully.

His kind of responsiveness raises the interesting educational problem of whether teachers may be justified in describing select skew approaches to physics to their pupils, not only to leave loose ends for innovative developments, but generally. It can be argued that the best informed financial patronage for research, and most valuable support for research programmes, is to be sought from managers and directors who understand a mixed conceptual language much more readily than would physicists with much stricter training in scientific communication.

A.R. UBBELOHDE
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Sea as dark as wine

SIR — When Homer sang of the "wine-dark" sea (see *Nature* **303**, 568, 1983; **307**, 590; **309**, 204, 1984) what he had in mind must surely have been its darkness, rather than the colour, and "dark as wine" might be a better translation. After all, one can say that "her eyes sparkled like diamonds", without implying that they were of the same colour.

Ionian Greeks knew all about wine, but with what other fluids would they have been familiar, to be compared in darkness with the sea? Blood would not have done, and the Homeric heroes had no use for ink, which was a muddy concoction available, if at all, only to scribes. So, gazing into the mysterious depths of a calm dark sea, how better could the poet then have described it, than with his simile of the transparent darkness of a full cup of wine?

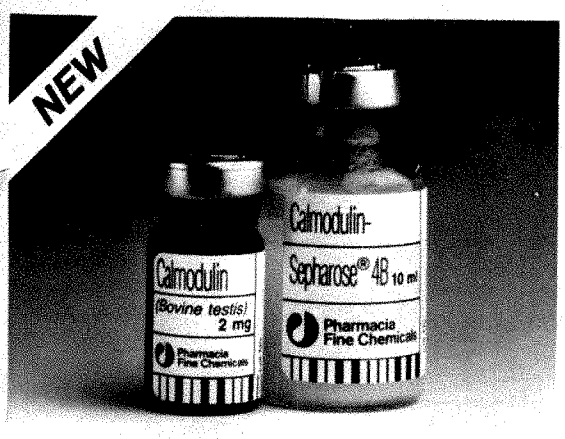
C.B. GOODHART
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Luddites defended

SIR — It has become fashionable to describe negative attitudes to technological change by making an analogy to the Luddites. Terms such as "neo-Luddite" or even "latter-day Luddite" are appearing regularly. The most recent I have noted is in David Sills' review (*Nature* 10 May, p.185). p.185).

The term seems to imply a senseless denigration of new ideas, an attempt to retain the old. I would suggest that this is a gross generalization. It strikes me that the original Luddite leaders were more concerned with the terrifying effects that new technology was to have on the workforce. Whilst their methods of protecting people from unemployment, with its associated evils, were violent and destructive, their intentions were highly honourable and deserving of our attention today.

RICHARD MORRISH
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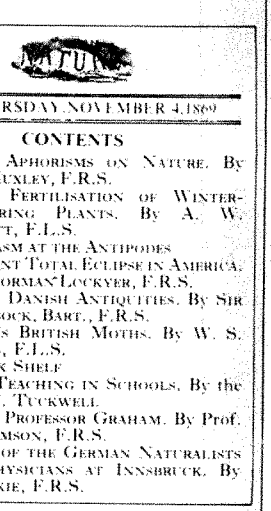
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Contrasts in near-similarity

The Low Countries, Belgium, Luxembourg and the Netherlands, are far from being the homogeneous corner of Northern Europe that geography suggests.

THE hallmark of densely populated Europe is diversity; the Low Countries — the Gallic name for the regions bordering the estuary of the Rhine — are Europe in microcosm. Socially and politically, the Netherlands, Belgium and Luxembourg between them embody all the features of a continent repeatedly reworked by twenty-three centuries of recorded history. Since the Roman pacification of northern Europe, the strategic importance of this patch of land has given it the unenviable distinction of being that on which the great European armed conflicts have been staged. The marks of those battles are still plain to see in every other rebuilt market square. The wonder is that so much remains of the distant past — cathedral spires glimpsed from miles away over flat alluvial farmland, the art treasures of many centuries and the indomitably cosmopolitan conviction that the whole of Europe can be run from the back yards of these northern fens.

The following pages will also show the Low Countries to be in microcosm representative of Europe's contemporary problems. Once comfortably prosperous economies are now, surprisingly, less so. Communal divisions rooted in language or religion are now openly contentious, with the result that tiny nation states are being further splintered into autonomous regions. Fierce public arguments rage (especially in the radical Netherlands) on questions such as the safety of nuclear power stations or the prudence of providing hospitality for other people's nuclear missiles. (Last week, the Dutch coalition government postponed for another eighteen months the decision whether to carry through its 1979 obligation to provide sites for nuclear missiles of the North Atlantic Treaty Organization.) And Belgium and the Netherlands must approach Europe's contemporary dilemma of how to join the new high-tech industrial revolution while keeping public expenditure to a minimum.

That Belgium and the Netherlands should be faced with such different problems is not surprising to Europeans, but may catch others unawares. In reality, the two countries have little more in common than their place on the map. (Luxembourg is something else again.) Historically, the Netherlands has the greater claim on public attention, perhaps because so many Dutchmen were quick to recognize the importance of what was happening in southern Europe during the Renaissance. Erasmus, part-philosopher and part pre-Newtonian physicist, was one of them. In the seventeenth century, van Leeuwenhoek did for the microscope what Galileo had done for the telescope, showing the potential of primitive pieces of glass. Huygens, whose physical insight made wave propagation through a medium a branch of mechanics, was a Dutchman. It remains something of a miracle that the University of Leiden should have served as a durable bridge between nineteenth-century physics and what came later through the work of H.A. Lorentz and his successors, notably Kramers. It is understandable that the government of the Netherlands should now be moving with great caution to impose external direction on the patterns of research in universities which have so recently been able to do so well.

Industrially, the two countries are also sharply different from each other. Mainland Europe's late response to the industrial revolution of the nineteenth century prompted quite different responses in the two small northern states. Both have made their way in the world by being industrious, but in very different ways. The Dutch seem to have concluded that it would be uneconomic just to make electric light bulbs simply for themselves and so Philips has made light bulbs for half of Europe. Now there are Shell and Unilever as well, all of them enterprises based squarely on a mercantile flair and the self-conscious application of research and development to the manufacture of products that people elsewhere will buy. Is this a continuation into the present of the old Dutch willingness to travel abroad or a measure of the strength of the Dutch universities? Belgian industry has taken a different route. Until quite recently, Belgium has been to northern Europe what Birmingham used to be to England, a conglomeration of small-scale metal (and diamond) workshops. (But now there are international pharmaceutical companies, as well as other people's multinationals such as ITT.) Is it that Belgium has had only modest expectations of what its universities can accomplish for its industry, at least until comparatively recently?

Such questions prompt over-simple answers. Since the Second World War, and with the unwinding of overseas empires in both Belgium and the Netherlands, it has been plain that each of them can function competitively only as a part of a larger European enterprise. It is therefore no accident that the Benelux economic union should have been a precursor of the European Communities. Each of them now gives outsiders the impression that it is marking time, waiting for the international climate to change for the better. The Dutch, meanwhile, have been flexible and ingenious in hitching some of their academic science to international enterprises, especially in fields such as astronomy. Belgium, with its language problem, is by contrast being forced along a path — separate pots of public money for separate linguistic regions — that cannot but be cramping for Belgian universities and, ultimately, for Belgian science. Can the mutual suspicion of the Walloons and Flemish be that important to them both?

So, it seems, the years immediately ahead are bound to be crucial for all three of the Low Countries. Will progress towards European integration make it easier for them to concentrate intellectual resources, and research and development in particular, on a smaller range of objectives without at the same time running the risk of neglecting activities that turn out, in the course of time, to be even more important? This, the recent dilemma of most European states in science and technology, is necessarily more acute in Belgium and the Netherlands than elsewhere. The Netherlands, with its established international connections, is better placed than Belgium, now bent on modernizing its metal-working industries and everything else in sight. Both countries would, however, benefit enormously from the more thorough integration of European academic institutions, universities and basic research establishments. Indeed, the problems encountered by Belgium and the Netherlands are a pointer not merely to a potentially fruitful area consistently neglected by European politicians (which will become more important as other states accede to the European Communities) but also to the way in which the rest of Europe does not fully share the benefit of the intellectual resource of these northern states. □

Science in the Low Countries

THIS survey of science in Belgium, Luxembourg and the Netherlands has been compiled and written by Robert Walgate, Chief European Correspondent of *Nature*.

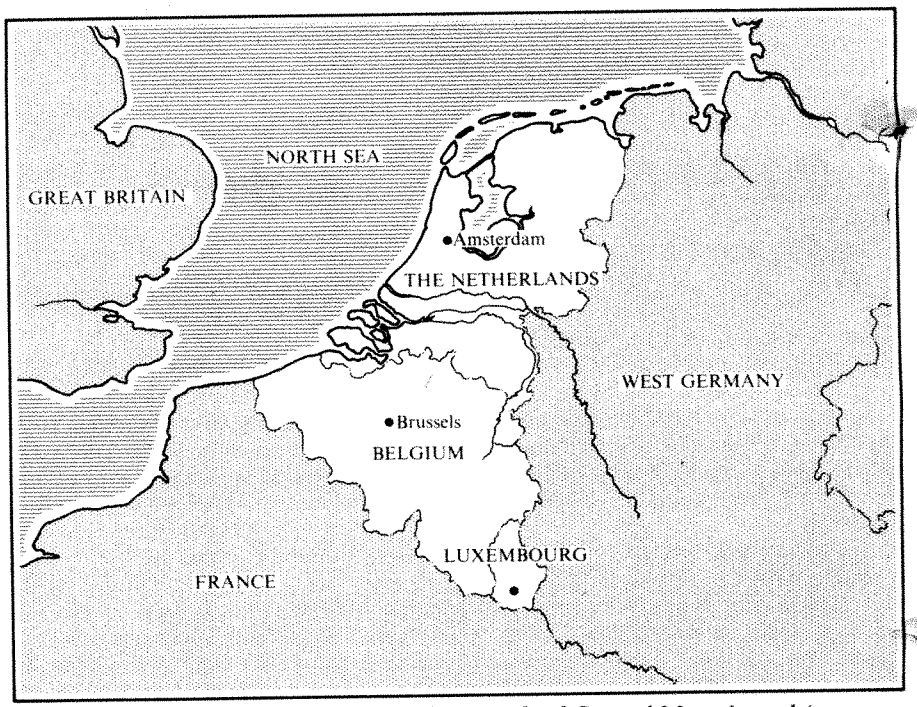
Geography

Small lands, big industry

ARE the Low Countries small? Some 14.3 million people live in the Netherlands; 9.9 million in Belgium; and 365,000 in Luxembourg: a total of a little more than 24 million people, less than half the population of Great Britain, France, Germany or Italy, and a tenth that of the United States. But their joint population density is three times that of France next door. And these people in their cabbage patches are highly productive: the Netherlands has probably the highest ratio of gross national product per square kilometre (£2.4 million) in the world.

Moreover, in the small continent of Europe, the Netherlands can claim to be medium-scale: politically, it chooses either to trail the big countries or lead the small, according to the policy it is pursuing. Smaller Belgium is further divided, roughly in half, by the constant regional sniping between Dutch-speaking Flanders in the north and French-speaking Wallonia in the south. Luxembourg, of course, is tiny, a little anomaly in modern Europe.

Geographically most of the Netherlands and the northern half of Belgium form the delta of the great river system of the Rhine, the Meuse and the Escaut rivers; and nearly half of the Netherlands (and more than half its population) is below sea-level (the windmills were — and are — water pumps, not flour mills). This gives both countries good flat agricultural land, and the Dutch, in particular, are renowned for their farming and agricultural science.



One Dutch university, Wageningen, is devoted to agriculture. Apart from that, the Netherlands has nine full universities, and three technical (engineering) universities. Three of the universities have religious affiliations: the Free University of Amsterdam (Dutch Reformed) and the Catholic Universities of Nijmegen and Tilburg.

Apart from agriculture and a strong dose of Calvinism (now forgotten, to judge by the drugs and flesh-pots of Amsterdam), in the past the Dutch have relied on sea-trade (which gives them an affinity with the English), and good business sense; but they also manufacture. In fact Holland supplies much of General Motor's steel (sea transport to Detroit up the St Lawrence is "almost free", they say). The Dutch also boast the headquarters of the multinationals Philips, Shell and Unilever, and are strong in the fermentation and pharmaceuticals business. Since the 1960s they have enjoyed a bountiful supply of natural gas from Gröningen in the north of the country, which some say has distorted the economy and the spirit of the Dutch.

Belgium has coal in Wallonia, and hence steel and the chemical industry — dominated by Solvay (which supported the historic Solvay conferences in physics). Flanders developed on textiles — for centuries it made cloth from British wool, and Belgian lace is famous — but it also has the almost independent offshoot of Bell Telephones, Bell Antwerp, flourishing foreign investment and is turning rapidly to new industries. It is thus outpacing the heavy industry of Wallonia, a circumstance which has now become a major cause of regional friction.

Belgium has six universities (three Flemish and three French) and eleven regional "university institutes". Two of the universities are Catholic (Leuven and Louvain-la-Neuve) and two free, that is neither fully state-controlled nor religious (the Flemish and French free universities of Brussels).

Luxembourg, finally, in the far south and hillier part of the region, has been predominantly a steel and metals town (half of its industrial employment was in steel 10 years ago, although the fraction is now declining and steel production with it). After steel, Luxembourg has rubber (Goodyear's European laboratory is there) and banking (it is like a little Switzerland to rich Belgians) and is now developing new industries. It has the first year of a university, and something approaching an academic hospital. □

Charlemagne's fondness for bathing

THE Netherlands, Belgium and Luxembourg are the northern tip of a corridor of states that once separated the Germanic from the French empires; and as rebellious buffer states, they have often been warred over — or upon. (It is appropriate, said one sardonic Belgian, that the North Atlantic Treaty Organization NATO has made its headquarters in Brussels.)

It is believable, although not necessarily true, that the blood soaked countries owe their existence to Charlemagne's love of a hot bath. This last great unifier of Europe was crowned in AD 800 and made his court at Aachen, now a German city but just over the border from the southern Netherlands. He chose that city, it is said, because as a lover of the good life he liked to bathe in its hot springs — there may have been some sound military reasons too, as Charlemagne was a soldier as well as a lover of baths, but these reasons are unclear. But for whatever cause, Aachen was the focus of his empire when he was planning its division among his sons, and Charlemagne seems to have planned the division symmetrically.

But even Charlemagne's plans could come adrift, and in the event, only one of his sons survived, leaving the empire intact for a generation. That son then enacted the division among his own three sons: one took the Germanic lands, so providing the early foundation of Germany; one took what is now France; and one was granted a corridor of land which ran from the low countries south through Alsace-Lorraine (now part of France) and on to the Mediterranean. These were the buffer states between France and Germany, of which the low countries were for long the marshy and neglected north.

The present states were first defined when Belgium seceded in 1830 from the United Netherlands (a product of the Napoleonic wars) after a revolution in Brussels; and second when Luxembourg won its independence from the Netherlands in 1890. In royal terms, they now form two kingdoms and a duchy (Luxembourg). □

Economics

The end of equality

ONE of Belgium's grander scientists, Nobel Prize winner Ilya Prigogine, is concerned — among other things — with a topic that proves strangely appropriate for this review of science politics in the Netherlands, Belgium and Luxembourg: the matter of "strange attractors".

These are states in phase space towards which complicated systems may converge, somewhat like a ground state in classical physics.

So Belgian and Dutch science, it seems — to leave Luxembourg aside for the moment — have converged along quite different paths towards the same strange attractor. Moreover it is one that seems to afflict much of European science: a kind of balkanization of science politics that ends up distributing government resources more or less evenly, or at least much more evenly than is good for the health of research — which, with some exceptions and caveats, often depends on the very inequitable principle of the best getting the most.

But "equitable distribution" has developed to an extraordinary degree in the Netherlands, where until very recently only 7 per cent of basic research funds in the universities went through a semblance of national peer review and where, of that 7 per cent, perhaps only a quarter went through a really serious competition for funds. Despite government attempts to introduce selection, and hence a kind of scientific aristocracy, as recently as last November a tongue-in-cheek science policy official said "we don't like to make kings and queens here". This was not treason against the Dutch Royal family: he was drawing attention to another honoured Dutch tradition, even distribution — a belief in a good solid mean value rather than peaks and troughs.

This Dutch situation seems to stem, perhaps as does much of the Dutch character, from centuries of cooperation to drain the land and keep out the sea; and it was emphasized by the democratic revolution in the universities in 1968. But it can also be seen as a Dutch version of a general European principle: that academics receive funds in proportion to their academic-political weight. (Perhaps that in turn stems from the long pre-scientific history of academic bickering in Europe.) It is evident throughout Europe that there are academic kings, and that — more than is the case in, say, the United States — kingship does not necessarily correspond to ability or productivity. In the Netherlands, this principle has merely been reversed. In the Netherlands, university democracy — and the very fear of kingship — has merely spread the king's rights over the whole academic community. This then taxes the government

Table 1 Gross national product (GNP) and population

	Working population (million)	Area (10^3 km^2)	GNP ($ thousand million)	GNP per head ($)	GNP per km^2 ($ million)
Netherlands	5.0	41	106	23,000	2.6
Belgium	4.1	31	79	21,000	2.5
Luxembourg	0.16	2.6	4	25,000	1.5
United Kingdom	26	244	400	15,000	1.6

These figures demonstrate the relative economic weakness within the low countries of Belgium, in terms of per capita GNP, but the strength of the whole region relative to the United Kingdom.

Table 2 National research and development budgets

	Government			Industry	
	Total ($ million)	% GNP	Defence component	Total ($ million)	% GNP
Netherlands	1,007	0.95	3%	986	0.93
Belgium	333	0.42	0.3%	698	0.93
United Kingdom	3,821	0.95	50%	3,423	0.86

This table again shows a relatively poor performance by Belgium — in terms of government support as a fraction of GNP, which as Table 1 shows was itself low relative to the Netherlands. Allowing for the small fraction of the low countries' spending on defence science, the Dutch research and development budget fraction works out somewhat higher than expected in an advanced country, and the Belgian slightly lower. There are no official science budget figures for Luxembourg, but an unofficial estimate puts government research and development at a miniscule 0.1 per cent of the Luxembourg GNP: most of the research there is applied research done by the steel and rubber industries.

Table 3 Division of government research and development budget

	Applied research in industry	Applied research in agriculture	Fundamental research	Human and social sciences
Netherlands	14%	8%	56%	22%
Belgium	35%	6%	41%	18%
United Kingdom	33%	8%	47%	12%

This indicates the low weight given to industrial research support by the Dutch government, and the relatively high weight given to the social and human sciences in both Belgium and the Netherlands. In the Netherlands at least, these sciences have been adopted enthusiastically by government, the administration, and politicians as an aid to policy-making. And the Dutch neglect of industry in fact reflects the great weight of the so-called "big five" companies in the Netherlands — including Philips, Shell and Unilever — which between them cover 70 per cent of Dutch industrial research and development. The big five have been thought, no doubt correctly, well-able to look after themselves. The neglect, therefore, has been of Dutch small and medium size industry; but the government has made and is making moves to correct the imbalance.

Table 4 University research funds

	University students	University research funds ($ million)	Proportion through research councils	University research as % of all national research and development	University research funds per student taught ($)
Netherlands	147,000	522	7%	25%	3,550
Belgium	110,000	223	27%	21%	2,030
United Kingdom	251,990	906	46.2%	11%	3,595

Here we calculate the research funds available (including the research proportion of academic salaries) per student taught — a figure which gives a measure of the research intensity of the universities compared with teaching. On this table the Dutch universities have nearly double the research intensity of the Belgian. This makes the Belgian universities look poor, but academics in Holland admit that their universities have been among the richest in the world. Other figures of interest: the small fraction of Dutch research funds that goes through research council peer-review, and in both countries the high proportion of university research as a fraction of all research and development (about double the fraction in the United Kingdom). These figures are based on the latest OECD science and technology indicators (1984) which refer to the year 1979. The figure of 46.2% for the proportion of UK university research sponsored by resource councils relates to 1980/81.

proportionally, in a kind of royal brotherhood.

The problem is only slightly easier in Belgium, where funds have been distributed more unevenly, and to some extent on scientific grounds — particularly since the institution of a prime-ministerial "top-groups' " fund in 1970. However, a large fraction of funds has been distributed among the nominally six complete universities according to what Belgians describe as "the theory of the four forces" — equal distribution between Catholic and non-Catholic, and between Flemish (the Dutch-speaking northerners) and Walloon (French-speaking southerners).

As a result, then, professors and researchers in both countries, in bulk and on the average, have received their money more through mere queuing, more through the essentially political demand of "fair proportion" than through scientific judgement.

However, Belgium and the Netherlands are not entirely without systems of selective funding, and the occasional watering of the best plants can and does have dramatic effects: as, for just two examples, with Prigogine's own laboratory, and astronomy in the Netherlands.

Moreover — and hence the headline above — things are changing in the Low Countries. The well-worn "economic crisis" is forcing drastic reappraisal of the equal-shares approach and, with very different mechanisms and effects, selectivity is increasing. The Netherlands is making the closest approach to extending peer-judgement in the basic sciences; Belgium is failing in this, and at the same time reducing its support of basic science. Both countries are increasing judgement at the level of national and — in the case of Belgium — regional politics. In this way, applied scientific and political criteria are encroaching very fast on the Belgian universities, faster than is probably advisable. These different approaches and their consequent problems are outlined in the following pages. □

The Netherlands

Shadow boxing with peer review

THE most fundamental change facing researchers in Dutch universities is not a loss of funds — though they face that too — but an increase in national refereeing of what funds there are. This threatens (or promises, according to your point of view) to change the whole basis of support for the bulk of basic research in Holland — and to put a new policy instrument in the hands of the government.

Until recently, over 90 per cent of the research funds received by the universities underwent no national review. The money was — and much of it still is — distributed at university level, by local research councils. These, with some exceptions, such as the Leiden council which has developed a method of selection based on citation analysis, find it difficult to make negative judgements about colleagues' work. And even at Leiden, such judgements are painful: because citation analysis can be challenged; because council members are judging the work of friends met daily; and because — given the small scale of a single university — often the work to be judged is in a discipline far from entirely understood by most council members. As a result, of course, money has been largely distributed through a system of courtesy rather than quality.

This may be gentlemanly; it may allow some rare lines of research to be followed that would otherwise be forgotten; but it leads to a loss of competitive edge, and it certainly does not suit times when money is short.

Hence the introduction of a system called "conditional financing", which offers a gold star — "protection" — to research projects that gain approval by national committees.

But for the moment, although many gold stars have already been awarded, no one really knows what this approval — or the lack of it — will mean. For the time being, the money comes to all much as before: the scheme has been one merely of labelling, the marking of a cross on the door. It is the time, in Dutch research, of the phoney war.

However, protection, says the minister of education and science Wim Deetman, does mean that a project will be exempt from further unforeseen cuts in university spending for five years. (Or at least, he says he will give that protection "all his political weight".) Moreover, the real selective war among projects is to come: it will begin in a year or two. "Research has finally become visible as a separate element in the budget", said a spokesman of the ministry of education, ominously, and in future "it could be re-allocated between the universities".

To some minds, this re-allocation, favouring some universities for research, is the whole purpose of conditional financing. But this runs hard against the Dutch tradition of equal treatment. A common plea is that the Dutch universities are really much the same: that there is no Harvard or Oxford. The fact is that there are variations, but no common pattern. Take medicine, for example: one university has an 85 per cent success rate in the final examinations; another has under 60 per cent. But, says Professor Veltman of the Technical University of Delft "It's very difficult to find extreme differences, because from year to year we've always made sure we've had the same amount of money, the same procedure for attracting professors, and more or less the same experimental facilities."

However, Veltman admits occasional differences in quality "because in some places you have a very good man, or a very strong team", and it is exactly this kind of difference that conditional financing will emphasize.

There is an unofficial target: that in any university, approximately half its research should be covered by conditional financing (that is, have won approval in the national committees), the rest coming unrefereed as before. But so far only about 30 per cent of university research has been subject to the scheme, so not enough research has yet

ZWO gives benefit of the doubt

THE Dutch national research council for basic research, ZWO, judged two out of three projects submitted by universities for the protection of "conditional financing" (see below), and rejected one in five. But they claim they were only "marginally involved" in the exercise.

ZWO is familiar with the distribution of grants awarded by peer review. But the council had to adopt a completely different selection procedure, and new committees, to assess the conditional financing projects, says its director Professor R. van Lieshout. ZWO had no role in defining the nature of a proposal; nor how it should be judged; nor even in what fields the council should judge. "In that sense we were only marginally involved". The minister's instructions were "too loose, too open" so the ZWO judgements were made "on someone else's terms of reference. . . Which were not clear and sometimes contradictory. . . "

The minister imposed three conditions, of which the most extraordinary was that the review should *not* look into a project in depth. Rather, as ZWO saw it, the reviewers were only to investigate a group's record, its potential, and whether its project was serious or window dressing.

But very often all the ZWO committees (specially convened for the purpose) could do was assess records, for the projects themselves were defined so sketchily that assessors could not decide whether they were good or bad. "We could not consider, as usual for a ZWO proposal, who was going to do what when, whether the project was well-planned, whether the researchers had the right contacts abroad, whether they even had the equipment — the review was much lighter. It was more a matter of trust, understanding, and giving the benefit of the doubt".

Nevertheless says van Lieshout "Very often the people on the special committees knew the groups, what they had done and could do. So they could detect tricks, and fill in what was not on paper. So that's why here at ZWO" — despite everything — "the conditional financing reviews worked quite well. . . ". □

passed through the scheme to start re-allocation. When this happens, in two or three years' time, universities that have gained few approvals will see their unrefereed research funds diminish in proportion, and to those that have more approvals, more funds will be given.

That is when the conditional financing fireworks may begin, not least because of a flaw in the whole system. The refereeing system for conditional financing was established in haste, and it is a rickety, untested Gothic structure, full of anomalies. The best and most obvious projects are getting through safely, but the middling and the unusual face a lottery. But the ministry will keep the system, based on *ad hoc* committees in a complex of different non-governmental institutions, and a set of ambiguous instructions.

According to the ministry, some things may be changed, but not the main structure. However, a spokesman admitted "We have to create more unity from subject to subject. Some judgements were prospective, some retrospective. . .". The ministry has been thinking of improving its guidelines. "There has been a problem of clarity in the decision-making." But "we do not think in practise there were real problems. . . There will be fine tuning."

However the whole exercise was so novel for the Netherlands, it is no surprise that the fine tuning will, in some subjects, be more like a complete change of channel.

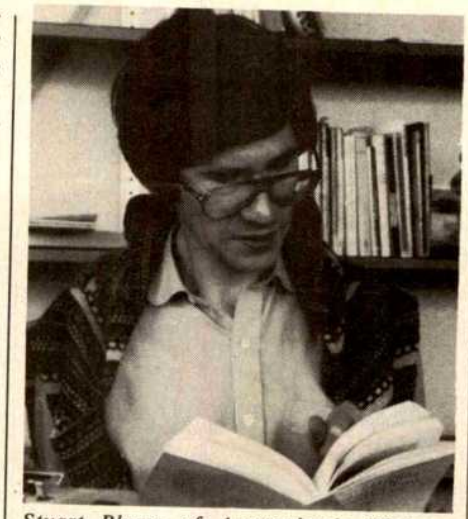
In chemical engineering, for example, projects were at least initially assessed by pure chemists with, as one engineer claimed, irrelevant ideas of what chemical engineering research should be. And in biochemistry, a relatively new group in carbohydrates at the University of Amsterdam

". . . there has been a problem of clarity in the decision making . . .

was judged, says a group member, on its publication record by a panel that knew nothing of the work or the group.

The group failed to win approval. But, says the aggrieved group, the panel should have used prospective criteria as the research was building up, and suffered from a "blinkered attitude" to the importance of carbohydrates in cell biology. Moreover, the group has set about proving its case by collecting hundreds of letters of support, and independent referees' reports on its work, from around the world.

And beyond such hearsay, the ministry of education, aware of criticisms, commissioned an external report on the assessment procedure. It was prepared by Jack Spaapen of the "Science Dynamics" group of the University of Amsterdam, headed by the English professor Stuart Blume, and details an extraordinarily varied and haphazard process of assessment. Spaapen studied the work of two assessment commissions from the basic research council, ZWO, three international *ad hoc* commissions, and commissions from the Royal



Stuart Blume of Amsterdam's "Science Dynamics" group.

Institute of Engineers (KIvI), the Royal Academy of Sciences (KNAW) and the National Agricultural Research Organization (NRLO).

All adopted "very different" procedures, which went beyond differences appropriate to tackling different subjects, Spaapen concluded. Some used committees to arrive at consensus; some "committees" never met, but interacted only on paper, with decisions taken by the chairman alone; some fed back criticisms to applicants and suggested re-application; some even invited applicants to the review. Writes Spaapen ". . . these problems largely derived from a divergence between the [conditional financing] procedure and usual evaluation roles. Uncertainty as to the precise function of their evaluation (and as to the consequences of a "no" in a given case) appears to have been common."

Perhaps as a result, rather few proposals were ultimately rejected (about 25 per cent), although the ministry claims that this was more than expected.

Another problem is the fear of increasing governmental control of the otherwise highly independent Dutch universities. The conservative government has not hidden the fact that come re-allocation, it may indicate some priorities in this great swathe of university research over which it previously had almost no knowledge and certainly no control. And since the financing involved is direct, and the assessment procedure so haphazard, since there is no organized buffer, no coherent policy-making research council, between government and spenders, the possibilities for control are great.

To government, the opportunity must be very tempting: but Professor R. van Lieshout, director of ZWO (which controls the 7 per cent of funds normally peer-reviewed) reacts very strongly to this idea: "It is of course a very dangerous development . . . My colleagues in Germany were quite shocked when they heard this. They said 'that's the way it started in the thirties'. They're very sensitive about such things there." In fact, says van Lieshout, there is no serious intention of such political control in the Netherlands, but "sometimes things slip in a certain direction even if no one intends them to; you have to be very careful." □

Universities

Cuts concentrate the mind

UNTIL the mid-1970s, Dutch university researchers were sitting very pretty. The rector of the technical university of Delft, asked if the Dutch universities had been the richest in the world, replied, laughing: "Oh yes!" Buildings, equipment and staff were all available as needed. In Delft, there were two technicians for every researcher, "a figure you will not easily find elsewhere in the world."

Then the oil crisis led to the first budget cuts. At first the cuts were mild. The government (then socialist-dominated) opted for "restrained budget growth" of 1 per cent per year in real terms. In the universities, this became a little harder: a real-terms freeze. But this was just play-acting compared with what was to come.

The new (conservative) government of 1977–78 faced inflation. It sought real cuts — and the university budget shrank by 1 per cent. But these cuts were only in buildings, materials, research equipment, and computers; there were no sackings, and — according to a government spokesman, the cuts posed "no problem". But gradually the knife came closer to the bone. The ministry decided not to compensate for inflation in 1979. Nor in 1980. Nor in 1981. Then the first real pain: over and above the "inflation penalty", in 1981 the universities faced a real budget cut — in personnel — of DFl 60 million (around 2 per cent, just over £1 million per university).

Then in 1982, the government demanded further, more sizeable cuts: another 10 per cent. Now the universities were in trouble. The ministry felt that savings could not be found on buildings or materials. The only place for further cuts was staff.

The ministry adopted three main principles:

- First, the cuts had to protect research. So cuts had to be found in education: in reducing the teaching hours per student. (Which, says the ministry, was a high figure in the Netherlands.)
- Second, the cuts were not to be even: said an official involved "we had to identify areas with the least and worst output: we had to make qualitative choices".
- And third, the present (right/centre) government decided not to make these choices itself.

So the problem was thrown to the universities, who had always pretended each

was equal to the other but now had to make distinctions among themselves — or face the minister making them himself. The bluff was called.

Yet it took the universities only 6 months, until March last year, to negotiate the 10 per cent cuts, and indicate where concentration (the elimination of departments at some universities, or their merging at another) could take place. This was fast work. So had there been substantial agreement?

"Yes", claimed one rector "but it led to a lot of trouble within the universities, as the democratic councils did not agree with what the boards had done . . .".

"No", said the ministry, the universities had fudged a lot of issues. Minister Deetman set himself the task of making sense of the universities' proposals, and of reaching decisions which were, his staff claim, "very careful, but on the other hand very clear". After long consultation, in November last year the minister offered each university a contract, covering the cuts they would suffer and a politician's guarantee of defence at that line.

This was it, said the minister: there would be no further changes. Two university councils (the university parliaments) told their boards not to sign: Amsterdam and Delft. But Deetman has threatened that he will simply not pay for departments to be closed under his plan. His list includes 34 departments, from the very small to the very large, which should be closed. Some departments, for instance geology at Leiden, are already closed.

Overall, the cuts aim to save the ministry of education some DFl 280 million (£70 million) from 1987. But on Deetman's orders, the cuts went a little deeper, reaching about DFl 310 million to provide a surplus of DFl 30 million for redistribution on new research projects. Some of the savings will go to biotechnology in Amsterdam; some will be used for a new pharmacological research laboratory in Leiden; some will go on psychology; and some on informatics, mostly in support of a ZWO (national research council) foundation in Amsterdam.

On the whole the government has protected the provincial universities; so the universities of Groningen in the north, and Twente in the east, were "saved"; they also had to economize, but less than the big, central universities in the "ring city" of Rotterdam, Delft, the Hague, Amsterdam and Utrecht where most Dutchmen and women live. As one president put it to this Englishman, "the general policy was that London can look after itself!"

The University of Limburg, for example, will actually get some new departments. "This is a political decision — I think that the academic Netherlands could easily do without that university" complains one "ring city" rector. Limburg is in Maastricht (in the southern tail of the Netherlands), rather distant from other universities, in an old coalmining region,



Although not faced with Belgium's regional troubles (see page 507), the "ring city" of Rotterdam, the Hague, Amsterdam and Delft is looked on with envy by "provincial" towns.

20 km from Aachen in West Germany. "You get the ridiculous situation that the most expensive academic hospital in the world is in Aachen, and the most expensive in the Netherlnds is in Maastricht, and you can see the one from the other — it's ridiculous. It's all to do with a decision to redevelop the area" says the complainant.

As for the three "private" religious universities, very little of the religious element is left in them but they have sometimes been able to make use of it during "concentration". It gives them a certain power, through religious connections, in parliament. For example the Roman Catholic university of Nijmegen was to lose Roman studies, but said this was impossible because it was RC. But the Protestant Free University of Amsterdam had the choice of saving sociology or dentistry, and they chose the latter, and were defended on the choice, whereas on religious grounds the former was more arguable. It thus appears that these universities simply use religion quite cynically, when it is useful to them.

In many ways, then, Deetman has achieved his aim — an uneven distribution of pain, although the unevenness has not always been defensible on academic grounds alone.

But the story of university concentration is not over yet. The next problems to face the minister will come in the courts, where the government must defend seven particular decisions. The most serious — or at least spectacular — concerns the justice of the closure of dentistry in Utrecht, the biggest dentistry department in the country and the biggest of all departments to be closed, saving DFl 20 million (£5 million). The closure has created much emotion, but the ministry won a first hearing of Utrecht's case in a lower court (in private chambers).

And there are still some other loose ends: in some subjects only the budget cuts have been defined: in sociology, economics, law, and medicine, including the academic hospitals, one of which — in Amsterdam — has been threatened. Deetman will decide in these areas this year if a department or two has to go. □

Degrees on the dole — early retirements

THERE are some 160,000 Dutch men and women with a degree. Of those, 15,000 are unemployed. University lecturers and professors are now adding to the figures, as budget cuts bite deeper.

The Technical University of Delft, for example, has about 4,000 staff, of which 1,400 are scientists. Some 600 people must go — and although the university can choose who should leave, strong university democracy will put greater pressure on the academic than the non-academic staff, the rector believes.

Professors themselves are not exempt, and as a further, nationwide measure, a whole cohort of professors must retire this year. The retirement age for a Dutch professor falls this summer from 70 to 65, to begin a 20 per cent reduction in full professorships in the Netherlands from 2,800 to 2,260 over five years. A total of 150 over-65-year-olds will be leaving in August, most of them not to be replaced.

But when it comes to redundancies, Dutch university staff benefit from being civil servants. The rector of Delft claims he often wonders whether he should take redundancy, because there are so many advantages. A redundant civil servant may draw 80 per cent of his or her final salary for a long period, and may supplement this up to 100 per cent through a full-time job while part-time earnings (such as consultancies) are ignored.

So the scheme is attractive — at least for well-established academics with good links with industry. But there is a drawback — government has been warned that it might not be able to pay, and that will have to lower the rates, or limit the number of years they pay. So, says the Delft rector, "that makes many people shy of retiring at the moment. . ."

And says van Lieshout, director of ZWO "I don't think it's a very nice prospect at 32 to be told now you can go home with 80 per cent of your salary, and you don't have to do anything anymore. That's not something to look forward to."

But the early redundancies are "a human disaster" according to the president of Leiden. People leaving are often too specialized to find work. "But if I look at the opportunity to cut dead wood . . . I think that in the long term we will have better universities." □

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Going shopping for science

If 1968 brought socialism and democracy to the Dutch universities, the 1980s are certainly bringing reaction. But, remarkably, one "socialist" innovation is surviving: the science shop. Science shops offer a modicum of university facilities (time, expertise, research) to anyone who cares to ask for it.

Typical shoppers used to be trade unions or environmental groups, but the range was never limited. The most common clients (in Amsterdam at least) now appear to be women's groups; the trade unions, while retaining interest in *ad hoc* questions, are pushing the shops towards more programmed, project-oriented work. (There is a project, for example, centred on electronic banking and its effects on employment.)

But science shops were once the leading edge of university radicalism: now they are commonplace. Every university has one, although the first (Amsterdam's) remains the biggest, and they are recognized and supported by the university boards and the ministry.

So how did they take root? More to the point, how was it that the shops' founders — the familiar 1960s rag-bag of left-wing science students and lecturers — did not dissolve into factionalism, as such groups did in almost every other country?

Loet Leyersdorff, one of the prime movers of the Amsterdam science shop, now on the staff of the Science Dynamics unit at the University of Amsterdam, believes there were several reasons, but most interesting among them were two: First, the trade unions gave very early support to the movement, and were keen on practical results. And second, majority rule in the shops' management. There is a strong tendency to compromise in the Netherlands, Leyersdorff explains, and this Dutch tradition is very strong in Amsterdam. "Even old-fashioned Marxists are ready to compromise." □



Posters at the "Science Shop" in Amsterdam.

Research council

Tiny budget — broad spectrum

THE Dutch have one research council, or coordinating body, to cover the whole of basic research — from the humanities to physics. Called ZWO, it is the only national body that allocates grants through thorough peer review (conditional financing aside). This gives ZWO a special prestige in Holland.

But ZWO has only a relatively tiny budget — covering, overall, about an eighth of Dutch university-level fundamental research. According to a recent survey the 13 Dutch universities spend around DFl 1,650 million (£410 million) on research. The ZWO is DFl 200 million (£50 million), about half of which is spent in its own independent institutes.

But there is overlap: ZWO institute directors often teach in universities. So there is a passing similarity between ZWO and the French national research council, CNRS. "There's exactly a factor 10 between us", laughs ZWO director Professor R. van Lieshout, a physicist from Amsterdam. "They have 23,000 people, we have 2,300, and there's almost the same factor in budgets. But they have many more institutes than us: for them, CNRS is practically only institutes; here it's 50/50."

But there is a cachet for a Dutchman in being funded by ZWO, as there is for a Frenchman funded by CNRS. ". . . we like to think so", says van Lieshout. "But it's not true in all fields", he says candidly. "For instance in social science some people think we're not helping enough and are funding the wrong things . . .".

For historical reasons, ZWO is most prominent in physics. There are five ZWO physics institutes, which consume half the ZWO physics budget; the other half goes to university groups. Of university physics spending, some 30 per cent comes through the ZWO. In some fields — such as plasma physics — there is work only at ZWO institutes.

In biology, the Royal Academy of Sciences (KNAW) plays an important role, mostly in the ecological sciences, but, says van Lieshout, the KNAW institutes are only loosely linked to university life, unlike ZWO institutes.

There are no biological institutes in ZWO, however. The biologists did not want them, it seems. ZWO supports about 20 per cent of all university basic biology, apart from its support for molecular biology through the chemistry division. (There are other substantial sources for biology through the agricultural university of Wageningen, through the medical research foundations.)

ZWO was founded in 1950, and began by integrating physics and mathematics institutes established four years earlier. The main aim was to tidy up the funding of basic sciences. After 1950, ZWO began to create other institutes on the model of the physics institute (FOM) and the mathematics centre. First to have institutes were chemistry and astronomy; and ultimately ZWO was spawning foundations in subjects such as theology, literature, linguistics and history.

Recently the budget has been "stable" — which in practise, because of falling university budgets, has given it an increasing, although still small, part in university spending. Last year the council won a small real increase (1.5 per cent) "through a technicality".

But next year the budget could go down. The finance minister of the conservative coalition government plans a further large cut in government spending. It is "almost unavoidable" that the cut will hit ZWO, van Lieshout fears "because they've cut the universities already, and protected their research through 'conditional financing'; so it's very difficult for them to go back and squeeze more out. They will be forced to cut organizations that haven't been hit yet. We're one of them." If there are cuts, the first item to be affected will be the support of research students, he says.

. . . in social science some people think we're funding the wrong things . . .

Neverthless, ZWO has optimistically requested an increase from DFl 200 million to DFl 204 million next year, partly to cover an increase in social science and humanities spending requested by the government; "Whether we can still have an increase for physics, medicine, biology and so on I don't know. We're trying to get one." ZWO has also requested a DFl 20 million capital equipment budget (on top of the DFl 200 million).

There should also be some money for ZWO in a recent, major government informatics plan: DFl 1,300 million (£325 million) mainly on education in informatics from the kindergarten to industry — "The whole population is to suffer from it!"

Has 1.5 per cent been enough to keep ZWO at the top? Probably not, says one ZWO staff member. A few years ago ZWO physicists tried to quantify what increase was needed just to keep up with new experimental techniques: they came to 4–5 per cent a year. But, says van Lieshout "Maybe our equipment budget was a little luxurious in the past here and there but it should not have gone down as far as it has."

ZWO needs to find cash to do something for LEP, for JET, to keep up its effort in astronomy, for apparatus for chemistry, and for a small computer or a computer link for the humanities and social sciences. ZWO is also seeking big equipment for research on surfaces: and would like to do joint research with the multinational

Astronomers spread far and wide

"DUTCHMEN live in a small country and we have to collaborate to get anywhere", says the chairman of the department of astronomy at the University of Leiden, Professor Harry van der Laan. Which may be one of the reasons why Dutch astronomy is so well known, particularly in the United States — where Dutchmen (mostly from Leiden) have headed many key facilities like the Mount Palomar telescope (for a long while the largest in the world), the astronomy departments of Yale, Harvard, and the US Naval Observatory (twice), and a Dutchman (Oort, previously director of Leiden Observatory) was ESO's founding father.

But Holland spends less on astronomy than France, West Germany or Great Britain, whether measured in proportion to either the gross national product or the population. And the country has fewer astronomers per head than the United Kingdom (the Dutch figure is comparable to the French and the German). The reason for the Dutch success, van der Laan suggests, is that Holland has had some great astronomers who have also created great Dutch schools (he mentions Kapteyn, Minnaert, and Oort).

Their students found Holland too small, says van der Laan. So Dutch astronomers colonized the world's telescopes and gained a reputation.

Moreover, in international collaborations Dutch astronomers have insisted that they win their share on the basis of research quality rather than any measure of funding, which given the size of the Netherlands would of course be small. In the collaboration with the United Kingdom over La Palma (in the Canary Islands) and Mauna Kea (Hawaii), under which the Netherlands pays a fifth the cost of various telescopes, optical and millimetre-wave, Holland pleaded strongly that it should not get a block allocation of 20 per cent. "I said we should compete on merit in the single allocation commitee — in which of course we will be represented by our scientists" said van der Laan.



Harry van der Laan — backing collaboration with Britain.

According to van der Laan the collaboration with Britain has been developing "beautifully", and has led to a "convergence" of the work of other universities in Holland. The Dutch universities are now all collaborating to build state-of-the-art instruments for La Palma, whereas previously their efforts were much more scattered.

As for ESO, which the Netherlands launched (the United Kingdom does not belong), "it has had a long, slow, start partly because of the remoteness of the place (the telescopes are at La Silla in Chile), partly the trouble of having many European nations working together". But according to van der Laan La Silla is already "a real state-of-the-art observatory, a fantastic observatory". "More and more" it produces the astronomy that it should.

ESO was born in Leiden: Oort called a meeting of the half-dozen most prestigious senior astronomers in Europe, with the idea of collaboration, and the result was the launching of ESO.

Van der Laan would like to extend Dutch cooperation with Britain and ESO. He would like to see Britain and ESO get together, for the later 1990s, to build the next generation telescope. It would be a $50-100 million telescope, very important as a complement to the Space Telescope "and much cheaper. The total cost of the Space Telescope is now estimated to be $1,300 million, so the next generation telescope on the ground in 15 million class will cost only one tenth as much and will be astronomically just as important (in combination with the space telescope). "Since we are partners of the UK and members of ESO, we'd be happy to be a catalyst" to such an agreement, said van der Laan. □

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laboratories in Holland (such as Philips, see page 501), with which links have been weakening over the past few years.

Ten or twenty years ago it was obvious that there was a healthy job market in Dutch industry, so people in universities were often inclined to do work that was relevant, says van Lieshout. Also the companies toured round the universities looking for talent. "So as I put it there was a lot of *contact* research but not a lot of *contract* research." For the past five years there has been less of a market for academics. There was a period when the universities took a dislike to industry; and now multinationals no longer seek to do all their research in Holland. So the links have weakened "and we must be very careful that they don't get weaker."

Beyond mere finance, ZWO is expecting a re-structuring of its responsibilities and management, in a bill with which ZWO is not entirely happy. But the draft law does give ZWO the right to do applied research, a right it has sought for years. A small, young organization (STW) that now supports applied research in the universities will come under the ZWO wing making one broader-based institution.

Why does ZWO want applied research? "Well first of all no-one organizes that properly." TNO (the big Dutch national applied research organization) works in its own institutes and not in the universities (except by occasional collaborations). But ZWO, on its foundation, was denied the right to do applied research because of the existence of TNO. "That was a mistake", says van Lieshout.

As for the rest of the ZWO restructuring proposals, all is not well. The government's proposal was submitted to ZWO, the Academic Council, the Royal Academy and the Council for Science Policy for comment. "But the government found the comments rather unexpected . . . strong and rather negative. Since then there has been silence."

The proposal is self-contradictory, claims van Lieshout. First, the government announced it wished to step back and not be directly involved with ZWO, while yet retaining some influence. But in the government's detailed proposals, influence increases. Van Lieshout is happy to accept influence "if there's open dialogue between us; it's OK for a scientist to take up a slightly different line of research that's more interesting to government but just as interesting to science."

Present government influence take the form of an official representative of the government on the ZWO board. He has right of veto; but he never exercises it. In the proposed reform, the government superficially appears to want less, no more than an observer on the board. Would this not reduce their influence? Said a ZWO man "They want to have their influence without committing themselves in the board of the organization. They believe they have more power as an autonomous partner."

"If you want to make a caricature," says van Lieshout "ZWO will think about science all by itself, and will come up with a proposal; the government will think about it and come up with its own proposal, listening to parliament and to public opinion; and then the two opinions, generated under their own steam, will be confronted and we will come to a consensus."

"Well our idea is that that is not a good way of doing it, and that it's better to meet several times first on a lower level before you come to these views — and only then confront. But the government thinks it better to have the two lines separated and only confront at the end."

And in the detail of the proposal, it turns out that the minister will have to approve "every detail" of the budget. At present, the minister approves only the total sum.

"Of course there was discussion, but there was no official seal of approval on our allocation of the money. Now there will have to be." If the minister disagrees, ZWO may have to make a new budget.

As a further restriction, ZWO would have to consult the minister over any new international agreements. And on international relations ZWO has a rather independent point of view. ZWO is reluctant to enter into generalized international agreements, and it has only three: for exchanges with CNRS (France), CNR (Italy) and the Royal Society (Britain). ZWO has been offered exchange agreements with, for example, the US National Science Foundation, but has decided against them because, says van Lieshout "that kind of agreement we can have anyway . . . we can send people anywhere . . . there's no obstacle. And the universities also can do it on their own. So sometimes we don't even use up all the possibilities under our agreements because people can find their way without us."

However, when ZWO finds there is already international cooperation going on — say among astronomers — then it is willing to set up an international umbrella agreement that will make the collaboration official and "keep the money flowing". That is how ZWO reached an agreement with the British Science and Engineering Research Council (SERC) in astronomy and synchrotron radiation, to share the cost and use of British facilities, "only after it was quite clear that there was something workable, a concrete and very well defined project that would fulfil its aims." Says van Lieshout, "we're very much averse to starting an agreement just to see how it works out."

ZWO has also managed to avoid paying large international subscriptions. So it does not pay directly for membership of the European Molecular Biology Laboratory, although the question of continued membership "comes up all the time" in the Netherlands. Equally for CERN, the European organization for nuclear research in Geneva. ZWO does not pay the CERN subscription either, although "CERN was very much in the balance a year ago in the Netherlands . . . but the minister went there and was convinced".

The European Space Agency (ESA) is also outside the ZWO budget. So is the cost of scientific satellites (such as the infrared astronomical satellite IRAS), which is covered by the ministry of the economy. But there will be no more national Dutch research satellites, the minister said: there is not enough interest in industry, so Dutch space scientists must concentrate on ESA.

And they will do it through ZWO, as the Dutch space research institute, with a DFl 50 million budget for the building of instruments, has just been put under ZWO control. The move is "purely administrative", van Lieshout claims. "But now we have the opportunity — if we want to and after a few years — to shift the balance between space science and other fields of astronomy. □

Science policy

Half a minister better than none

AFTER a ten-year experiment with a science policy minister, the Netherlands now has none. Or perhaps a portion: in the present cabinet, the function has been combined with education in the post of minister of education and science, now held by the scourge of the Dutch universities, Dr Wim Deetman.

It was not a conscious decision to demote science policy — just a way of balancing the books of a delicate coalition of parties. But, says Mr von Spiegel, secretary-general of the directorate for science policy (which used to be the science policy ministry): "In Holland politicians are not interested in science."

Arguably as a result, and while Deetman was preoccupied with streamlining the universities, his captainless science policy

In Holland politicians are not interested in science. . .

directorate last year lost half its operational budget (originally DFl 70 million, som £17 million), a flexible fund for direct stimulus of research and development, and one of the directorate's most cherished instruments. (Otherwise it merely coordinates budgets under the control of other departments and advises on science policy.)

The loss occurred in a split which gave the directorate's work in "technology policy" to a new office in the ministry of economic affairs (which deals with industry). The new office also took on 10 of the policy staff. Thus von Spiegel and the remainder of his team find themselves rather exposed and isolated. And not only is the directorate preyed upon by the ministry of economic affairs, it is also at odds with the more bureaucratic department of eduction with which it is now combined. (The education department insists, in particular, on university autonomy, and even in the recent cuts largely allowed the universities to decide for themselves what to cut and what to defend. The science policy department style might have been different, but it was not allowed to play a significant part in the excercise.)

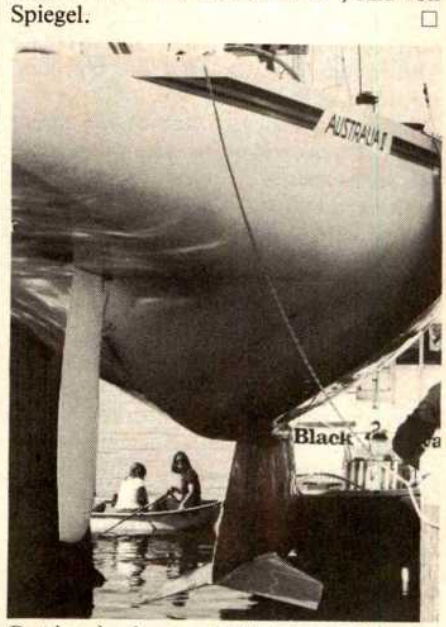
The science policy department is therefore being forced back into a more reflective role: "We will be less busy with operational tasks, with execution, than before", says Tindemans. The directorate will concern itself with the long-term rather than the short. But it retains its powers to coordinate budgets, and the minister of education and science is able to use the department to establish science and technology policies that cut across departmental boundaries — such as information technology, where the department has recently established a major new government action. That is a subject "of very broad importance", says Tindemans, bearing on the economy, education, health, public policy, and administration. Hence it made sense to give the definition of an informatics policy to the science policy directorate.

The directorate's DFl 300–500 (£75–125 million) informatics plan makes proposals for the expansion of informatics education (at all levels); offers funds for research, especially in software, and for applications — for example the computerization of Dutch records of physical infrastructure (telephones, cabling, pipes and so on). It also deals with networking and "task differentiation" (somewhat difficult in Holland) between big centres and small ones, involving the introduction of big computers like Cray 1s and Cyber 205s, where Holland has little experience; and deals with the transfer of academic expertise to industry. The acceptance of this report has been a science policy directorate success.

Also, the directorate retains control of the applied research council, TNO.

Intervention on departmental budgets and policy "will take more of our time in the future", Tindemans cliams. But before the departments quake in their shoes von Spiegel's view is that such intervention would prove very difficult.

The directorate is also managing an exercise to raise efficiency, and so cut spending, in some 60 government research institutes. Universities are included, but only as a point of reference. "We are looking at staffing and management of the centres" said Tindemans, in order to have an impact on the government budget for 1985. The savings are to come to DFl 130 million (£32 million) or about 5 per cent of the budget, less than the 10 per cent asked of the universities. "We think we can do it", said von Spiegel. □



Dutch technology was behind the revolutionary design for the hull of Australia II, which took the Americas Cup from the United States last year.

Biotechnology

Ambition and a track record

THE Netherlands is planning to make an "explosive impact" on biotechnology within the next couple of years. And they may well do so, not because Dutch companies can lay claim to the world's first genetically engineered vaccine (for pig and cow diarrhoea), and the first monoclonal diagnostic kit (which they can) but rather because the whole of biotechnical Holland is now, as one biotechnologist puts it, "in the start position".

The keys are context, and scale. First, Dutch biotechnology is forming within a coherent, flexible, apolitical, imaginative and believable national "strategy" (to call it a plan would make it sound too centralized and deterministic), in which basic research has a firm place. Second, although in a larger country such scheming can fail through excessive scale and, in particular, poor communication with industry, Holland is small enough that it really has a good chance of creating "The Netherlands Ltd". (History and geography have made cooperation natural to the Dutch.)

The strategy is the effort of a small group of biologists and industrialists, the national advisory committee on biotechnology, which has been giving direct advice to the Dutch cabinet. Its chairman, the most unacademic of academics Professor R.A. Schilperoort, plant geneticist of the University of Leiden, says the committee will wind up in May 1985, its work complete. The first real marketable products will emerge from the committee's Netherlands Ltd, through the big companies, within one or two years, Schilperoort believes. One of the major product areas will be in waste-water treatment, he expects.

From a practical point of view a university can't keep secrets . . .

The committee has asked the big Dutch industries in the area (including Shell, Gist Brocades and Unilever) what research they would like to be done in Holland, and who they would like to do it. "All the companies are showing great interest. It's very delicate — companies like to be very confidential." But through individual, one-to-one conversations largely with Schilperoort's aide, who has signed a secrecy agreement, the advisory committee has got to know, at least in outline, all the Dutch companies' plans in biotechnology. This makes sensible strategic planning on a national scale a reality — whereas in France, for example, similar moves by the socialist government were met largely by non-cooperation from industry.

One problem has been the predominance of projects initiated by scientists rather than technologists, but there are moves to change this. The idea, Schilperoort explains, is to orientate university and government laboratory research "a little bit more to the market." University research may be as fundamental as scientists wish but "It shouldn't conflict with research in industry . . . we want to make it a source of results and people."

The committee does not want to create a biotechnology centre, believing that such places grow "sleepy". "Instead we make centres on certain themes, which have to compete all the time for funds." Thus the committee has somewhat by-passed the national applied research organization, TNO, which might on the face of it have been the focus of the biotechnology programme.

"TNO got a substantial part of our money, we have stimulated their programme in biotechnology, but they will have to do better if they want to get more . . . . It's grown too big . . . it's surprising . . . TNO was thought to be more commercially oriented than the universities . . .".

On international collaboration in biotechnology, the committee has an unusual, Europeanist attitude. "We like to collaborate in areas in which we are strong. So we've chosen three areas for collaboration: agriculture, waste treatment and pollution, and process technology. It doesn't make sense to collaborate from weakness. This is nationalistic. Collaborating from strength then we have a chance to be a strong continent. If we only act at weak points we will never be strong!" This, says Schilperoort, "is our signal to Europe". Bilaterally, the Netherlands is making plans with Switzerland and Germany, and is in discussion with France and the United Kingdom.

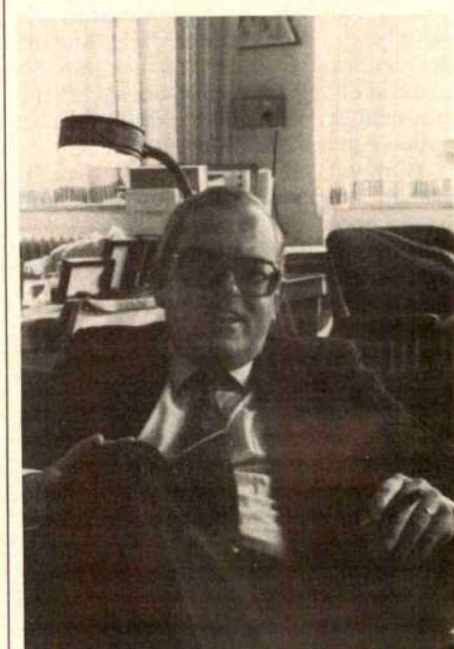
But back home, how far will this programme protect basic biology in the Netherlands against university cuts? "We are quite successful . . . there are really severe cuts, but from the money we have now we can support a lot of research." The committee itself has DFl 80 million to spend this year, and there is another DFl 30 million to be granted through a special priority-area committee. "We are making corrections to the cuts in the area of biotechnology."

And Schilperoort, who has had a strong personal influence on the development of the Dutch biotechnology programme, believes in basic research: "Our basic philosophy is that we need excellent, well-organized basic research — otherwise you are out . . . . It's very important to stimulate the creativity and originality of fundamental scientists, because you can't predict what they will find; we should only say, why not try working on organisms a little bit different from *E. coli* and see how far you can go . . .".

As for posts — "that's very important" — the committee would like to create university positions, in collaboration with the ministry of education; but only in the areas that will underpin industry's priorities.

There should be no secrecy in the universities, the committee believes. Fundamental research should be open research, says Schilperoort, both on principle and in practise. "You can't oblige a member of a university to sign a secrecy agreement . . . who will pay the money if he is sued? . . . from a practical point of view a university can't keep secrets."

But this is where the government applied research centres, like TNO, can come in. There, there are no problems of secrecy. So industry could transfer a successful, basic university project to the applied centres for development. "And we are thinking about how to set up small companies, seeing what mistakes we can avoid . . .". In the small entrepreneurial companies established abroad, market research has often been weak, they have begun without clients, and many companies will just fade away.



Chairman of the national committee, R.A. Schilperoort.

The Dutch programme is coming after the first wave of biotechnical excitement, but this very fact may give it an edge. There are many markets left, Schilperpoort believes. And it is the large companies which will lead the way. And they are prepared, having taken the biggest, most expensive scale-up steps — "much more expensive than a small amount of research". For example, Shell and Gist Brocades have created a joint, DFl 300 million, biotechnology laboratory.

Such things, plus good links with the universities, will create the Netherlands biotechnology enterprise. "Our Dutch industry is prepared . . . . They have good projects already and there needs to be a good, continuous, realistic flow . . .". And that, the committee is creating. □

Philips

Too big for a cautious Europe

By far the largest research organization in the Netherlands is not public but private: the 2,000-strong Eindhoven laboratory of Philips, the electrical equipment multinational. And beyond Eindhoven, Philips, the maker of the technically brilliant but in marketing terms unsuccessful Laservision, the silver disk, also employs a further 2,000 researchers overseas in England, France, Germany, Belgium and the United States.

Philips' research budget of DFl 500 million (£125 million) is more than double that of the Dutch national research council (ZWO). Its development budget is DFl 1,500 million (£750 million), supporting a product range that began with light bulbs and now covers almost everything electrical outside power engineering. The company is also the biggest employer in the Netherlands. Some 22 per cent of its employees are Dutch — but only 7 per cent of its sales. Eleven of its 12 divisions are managed from Holland, but its directors see the company as primarily European, with a quarter of its sales, half the factories, and two-thirds of its employees in Europe.

Philips' greatest single research strength is in materials, claims Philips vice-president for research Dr A.E. Pannenborg. The original lamp research was the investigation of materials under extreme conditions. From there Philips invented ferrites, had a flying start in transistors, works now in ceramics, metals, glass, fluorescent materials, semiconductors. In fact, breadth is one of Philips' greatest strengths — the development of Laservision embraced lithography, precision mechanics, optical technology, servotechnology, plastics and more. But yet, Philips is broadening still, by setting up a joint venture with the American communications giant AT&T on digital switching, aiming first at the European market. The objective: economies of scale in the development of equipment.

In telecommunications Philips' markets were not big enough. Inevitably, perhaps, the company has done well in the Netherlands, "and we've filled Saudi Arabia to the point they've got telephones on every palm tree", says Pannenborg, . Philips also has a market in the old Dutch colony of Indonesia and a modest position in Peru. "But if you add up our markets, we can't bear the costs. We were very happy that AT&T was available. We are complementary." AT&T has marketing in the United States, and Philips has competence in the worldwide CCITT standards, which the enormous American market has previously been able to ignore.

Cooperation with other companies in Europe, however, is developing only slowly. But the European Commission exercise on coordinating European research on information technology, Esprit, was a big step forward.

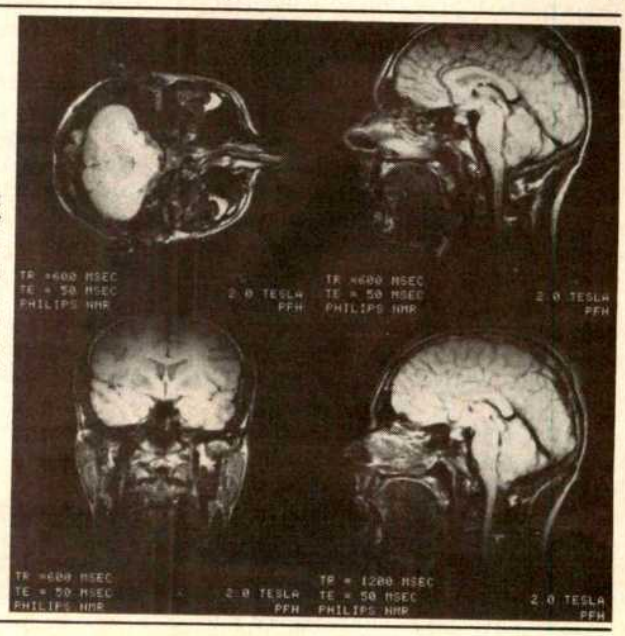
On academic cooperation, Philips' research works almost independently of the Dutch universities — except for staff, which are recruited young, spend 5–10 years in the research laboratories and then move into the product divisions. The average age in the Eindhoven laboratory is rising a little, but there is a firm policy of moving people on, in order to be able to recruit up to 200 graduates a year.

Nevertheless, Philips does not go in for contract research with the universities, or other outside laboratories in Holland. There were more links before the university "revolution" of 1968–69 but then the company pulled back — as did the universities at horror in involvement in matters commercial — and since then the two sides have been drifting further apart. There is now a new climate of willingness in the universities to cooperate, but, says Pannenborg, "The quality of university science is fair but not particularly good in this country." The "egalitarian wave" of 1968–69 led to a reluctance to develop centres of excellence. So there are very few places in Holland with which Philips would really like to cooperate. On the other hand, in computer aided design Philips has "intense and effective cooperation" with the Flemish university of Louvain over the border in Belgium.

. . . most of our competitors have died in the struggle. . .

But despite its arguably world-class research effort, which to some extent justifies its disdain of Dutch university research, Philips profits remain, to quote vice-president Pannenborg, "unacceptably low". The problem, he says, is less with Philips than with the conservative, unadventurous European customers. If Philips in Europe is healthy, says Pannenborg, then Philips is healthy worldwide. But in Europe "we lack the leading-edge customer. It makes life damned difficult!" Thus the true strength of America's Silicon Valley, claims Pannenborg, is not that there are so many highly competent silicon foundries, but that these foundries "are embedded in a place where you have the leading computer manufacturers, the leading weapons systems people, the leading automation people of the world who *suck* the products out of these foundries". By contrast the weakness of Europe is the cautious, reticent, non-innovative, wait-and-see attitude of the customer, whether institutional or individual. Nevertheless "if you look around Europe, we've done quite well. In fact most of our competitors have died in the struggle."

However, according to some outside observers Philips also suffers from a number of structural problems, the first being the separation between the research and product divisions which (say the critics) has allowed research to get carried away with its own ideas. Philips claims to have cured that problem recently by introducing more interaction. And some in Philips will admit that, perhaps, the quality of Philips' marketing does not match up with the quality of its research. Philips could do with a strengthening of "strategic marketing" — the kind of thing that might have taken into account at an early stage that the consumer was not going to be too interested in a video disk (Laservision) that could play but not record, however beautiful the technology, when it came on the market long after recordable videotape, and at a higher price. Philips is now working on home recording onto the disks, but there is a problem of stability of the record, and marketing tactics are now having to shift to the much smaller "professional" market. But perhaps, with a little more awareness of real markets, and a little less complaining about the backwardness of European consumers, Philips still has a bright future. □



THESE images of an adult head are another product of the Philips research effort. From the laboratories in Hamburg, West Germany, they represent the first 2-tesla images of the human head by nuclear magnetic resonance imaging.

Belgium

Universities poor and divided

UNIVERSITIES everywhere are struggling for money and posts. But the six full Belgian universities can claim a certain notoriety — as an extreme case.

The chief administrator of the national research council (FNRS) claimed recently that Dutch researchers have twice as much money for research as Belgian, and the French four times as much, and although "money for research" was left undefined, figures do show, for example, that Belgian university research funds, per undergraduate student taught, are less than half those in the Netherlands (see Table 4, page 493).

The best basic research survives, much of it only by shifting emphasis heavily towards the applied sciences. The national government has been supporting applied science with fashionable zeal — although some responsibility for applied science has been transferred to regional governments established in 1980. (One of these, Flanders, is taking up the task with particular excitement, with Wallonia a close second.) But in ten years there will be no new science in Belgium to apply, say many voices, as the basic sciences are more and more neglected . . . .

Another course for certain lucky groups is to hang on to an unreliable lifeline thrown by the late prime minister Théo LeFévre back in 1970. This is a prime-ministerial fund for "excellence", the "concerted action" programme (the "concert" is with the government). The programme, as conceived by LeFévre, offered temporary relief to important groups whose costs were rising too fast for the standard funding mechanism to cope. (Their budgets — even those of FNRS — are keyed to student numbers.) But temporary has become permanent, and many important groups now rely on concerted action funds to do their basic work; while, nevertheless, the continuance and allocation of the funds is unpredictably tied to government policy.

Yet despite the troubles, Belgian science is strong: molecular biology, in particular, has a worldwide reputation, and can boast two Nobel Prize winners since the Second World War (of which Christian de Duve, the discoverer of lysosomes, is still active with his international Institute of Cellular Pathology in Brussels). Physicist Ilya Prigogine is another renowned and energetic Belgian Nobel Prize winner; he also works in Brussels.

But there must be a question as to whether such success could ever recur in present conditions. The big slide began in 1976, when the Belgian Government began to seek savings on university spending. At the University of Ghent, for example, 20 years ago salaries accounted for only 70 per cent of income, with the remainder used for research. (This was the so-called "first stream" of research support, distributed within the university by a local committee as in Holland.) But now some 90 per cent of the budget goes in salaries; so the flow in the first stream has slowed by a factor of three, to a mere trickle. Said Professor Jean Brachet, biologist at the Free University of Brussels, "I was once on a committee here distributing money for apparatus. But now I'm told it's almost useless to go to the meeting because there's so little money to distribute."

As for posts, the universities face, since last year, a demand to make 10 per cent cuts in academic staff over the next seven years. According to Ghent plant geneticist Professor Marc van Montagu, in practice his faculty has made no permanent appointments at all for the past two years. (The same is true at the Catholic University of Louvain-la-Neuve, according to the dean of science there.) Says van Montagu: "Now we have to handle the university like a high school: if we're short of a biologist to teach a course we have to ask a chemistry teacher to do it: we're reaching that level... The problem of jobs is hitting professors as much as assistants."

And to add to their relative poverty, the universities suffer a special Belgian pain. Like all Belgian institutions, they face increasing rifts and tensions between the Dutch-speaking north (Flanders) and the French-speaking south (Wallonia). Four of the universities even make bad worse, as they suffer from a further historic division between Catholic and non-Catholic. As a result Belgian academic funding is all but divided into four equal pieces. Certainly it is divided in two — Flemish and Walloon — thus hindering the selective distribution of meagre resources. An exercise such as in the Netherlands, where the universities were "concentrated", departments closed, and people sacked — according to what were at least nominally judgements of quality — would be inconceivable in Belgium, as it would be far too inflammatory in regional or denominational terms.

Further, while the mobility of academic staff throughout Europe is bad, mobility in Belgium is nil. A budding researcher who arrives at a university at 18 is likely to stay there for life — if he or she is lucky enough to get a post.

But to take a somewhat Machiavellian view, there is one good side to all this: the near-anarchy has allowed certain pragmatic researcher-princes to establish their city-states. Just as princes raise taxes wherever they have influence, so do these researcher-princes raise funds; and the luckiest, most pragmatic and most persuasive may thus have been able to accumulate a greater relative income than they would have done if policy were more centrally controlled. Thus there is a greater presence in Belgium of centres of excellence than there is in neighbouring democratic Holland. □

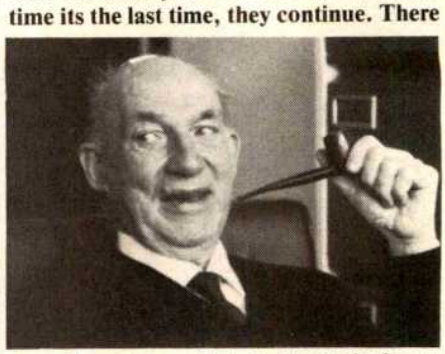
Actions concertées — lifeline of the strong

IN the 1960s Belgian basic research was funded well enough through the university budget with grants from the national research council (FNRS). But towards the end of the decade molecular biology, and some other sciences, outgrew these funds — and Prime Minister Théo LeFévre started a fund, dubbed "*actions concertées*" or "concerted actions".

The grants, beginning in 1970, were substantial and highly selective: the aim was to support excellence and only the strongest groups received them. They lasted five years at a time and could reach BF 10 million a year (£125,000), compared with university funds which were rarely more than a tenth of that for supplies. One microelectronics laboratory — at the Catholic University of Leuven — increased its size tenfold in this period.

But will they last? Staff at a key laboratory in molecular biology, at Rhode St Genèse near Brussels, founded by Professor Jean Brachet, have their fears. Brachet has retired, and now the contracts are decreasing; "and we are told that in two years they will finish". According to Brachet himself "the *action concertée* has not been the main source of funding for our applied projects, but in a field like embryology, when the *action concertée* is finished it's the end."

However, Brachet is not too perturbed: the science policy programming office of the prime minister that distributes the grants "say they want new projects — but I've been involved before in helping distribute the money and while they say every time its the last time, they continue. There are not so many groups of this size and quality in the country."



Jean Brachet — optimistic despite budgetary pressures

Incidentially, Brachet also met the famous LeFévre, who many in Belgium regard as having saved basic science. "He realized", says Brachet, "that to get people into research of any kind was an investment for the country, even if in the long run he was interested in boosting applied research. □

Industrial research council

Basic PhDs recipe for success

IRSIA, the Belgian national council for the stimulation of research in industry and agriculture, is a good deal more than it might seem from its title. Contrary to expectation — given Belgium's current political attachment to applied research — IRSIA plays an important role in maintaining the fundamental sciences.

Universities depend on IRSIA for research studentships, even in the most basic sciences. (IRSIA merely limits the field to "science", in the English sense of the word.) The council provides 600 such grants a year, awarded on the basis of interviews, compared with the mere 100 from the national (basic) research council, FNRS, which are offered to those with the best marks in first-degree examinations, and cover all academic disciplines.

IRSIA conducts 900 interviews for the studentships at its headquarters in Brussels every year. Apart from university assistantships, which are few, IRSIA claims to cover 80–90 per cent of students working seriously for a PhD.

But why should an applied research council take on such a job? It seems the object — which dates back to the council's post-war foundation — is to provide research-trained scientific manpower for industry. And IRSIA's view is that 'research' requires a certain state of mind, a certain approach, that must be learned before a student considers application.

It is possible to do a PhD in Belgium with grants paid neither by IRSIA nor by FNRS, but by industry. But, says IRSIA's director Dr J. van Keymeulen surprisingly: "this is not the best way to do it: then you have to produce some applied results . . . but a PhD is something else . . . an education".

All IRSIA asks of a professor proposing a candidate for an IRSIA studentship is that he or she must guarantee that the subject will lead to a doctorate. IRSIA claims to take no control over the subject matter of the PhDs, although this occasionally requires a little heart-searching. For example "we asked ourselves several times what the students of Professor Prigogine [Nobel Prize winner in irreversible thermodynamics] would do after that training in theoretical physics. But none of his ex-students is unemployed! So their training was very good because their scientific environment was good — not only Professor Prigogine but his co-workers also. I don't say the subject is of no interest — but it is important on the second level. The environment in which you live — that gives you your training."

Nevertheless, the system might not have survived if the studentships had not also acted as IRSIA's antennae in the university system. "They give us a very good idea what is going on in the university laboratories". They account for only 10 per cent of IRSIA's turnover; but the rest consists of various forms of aid for industrial and agricultural research, much of which finds its way back through joint projects into the university system. There, the studentships help IRSIA management keep in touch with who's who and what are present research trends.

But is all this not just rationalization of a historical circumstance, that the university PhD system began to depend on IRSIA grants? Is it truly a policy? "That takes us back to 1946", explains van Keymeulen "when IRSIA was created out of the FNRS. FNRS's job was mainly to sustain professors. . . We aimed to sustain projects in industry and agriculture. And we gave scholarships. (We started with 10.) We needed the manpower: Belgium was really underdeveloped in research and development. All we had was universities. The IRSIA idea was to create something in industry — and we needed the people." This historic role has become bigger and bigger.

But was it never the role of FNRS? "No. They selected the very best people. . . the candidates for Nobel Prizes. We had another group. We said we wanted good people, who could give good service to industry. That was our role." □

New studentships plus job in industry

THEY are being dubbed the "Maystadt boys" — although it is an equal opportunity offer. Science and budget minister Philippe Maystadt has established a budget of BF 900 million (£11 million) intended for younger students, just out of university (or having just finished a PhD), to work in fundamental research and learn techniques that would or could lead to industrial application.

Each grant lasts a maximum of three years. But if the person is hired by industry within that period, the laboratory has the right to hire someone new, again for three years. If the person is not hired by industry, they lose that position.

These are not postdoctorates — nor PhDs, although it is not excluded that a doctorate could be earned — but research training exercises lasting 1–3 years. Students on the biotechnical part of the programme, for example, have to learn the techniques of the kinetics of immobilized enzymes, or cloning or other techniques.

"It is a very intelligent programme", said one laboratory director just after a telephone call telling him he will have five of the posts to distribute. "It's really useful, as you will be able to get on with your own work on the condition that you give training to someone in basic techniques." And Maystadt's project, in fact, covers a lot more than biotechnology — a whole range of scientifically and technologically interesting fields.

However, not everyone is so pleased with the idea. The students must be young — they must never have had a job before. "And", said another recipient "in every contract we get nowadays it says that any result that derives directly or indirectly from the work will belong to the Belgian state. These people get BF 100,000 a year, about a third of what they need to work in genetic engineering, just for materials, so you have to find another two thirds. But each source of money says that everything that will be done belongs to me. . . And in some cases in these grants they forbid publication."

"But — these are 200 positions, and that must be basically good. . ." □

Research opportunities

No room for manoeuvre

IT is still possible to do a PhD in Belgium, although the number of grants is falling. But at the postdoctoral level? The constant refrain is that there are almost no postdocs in Belgium.

Universities can create permanent assistantships, but a law of 1976 restricted the number to below 40 per cent of the total number of assistants; and most universities have already exceeded that figure. "We have had no new posts at Louvain-la-Neuve for two years — at any level, in any faculty", according to Louvain-la-Neuve's dean of science, Pierre Macq.

At the same time, fewer students are interested in staying on for a PhD. For example, in 1983 a dozen new graduates from Louvain-la-Neuve sought research studentships from the national research council, FNRS; but in 1984 there are only four applicants. "It's a general situation: people don't believe in the future. The funds are going down but the demand is going down also", says Macq.

In Belgium, there is nothing like the French CNRS, or the British research councils, to provide postdoctoral posts outside the university system. FNRS has no laboratories. It has a few coveted permanent posts, but these — intended to be stop-gaps but already 12 years old — are almost vanishingly few and offer no career structure. To compare with the French CNRS, there is no level of 'director of research' which "makes life 'uneasy' for an ambitious young scientist", says Brachet.

Even so the competition is "incredible" for these positions. Says Professor Nicolas Glansdorff, a Brussels microbiologist "It has become almost impossible for a good — let's not say excellent — researcher to get one of these posts. If you look at the curri-

culum vitae of the winners you would say that most of them should be professors — and more than that for some of them. The problem is that in Belgium where the scientific level is quite good, we have each year maybe 15 people who've published in *Nature* and *Cell* and *PNAS* competing for one post! It's completely crazy."

So the unlucky ones in Glansdorff's laboratory — some of them in their 40s — live year by year, go on unemployment benefit for a time, come back again — "it's really traumatic for them".

At FNRS, these life fellowships are described as "the *nec plus ultra*". Yet they were introduced only as a 'parking lot' when university posts began to tighten up in the early 1970s. It was intended that universities would absorb them when the situation eased.

But the situation never did ease. "Now we have a completely new and very difficult situation", says Brussels engineering professor André Jaumotte, who was FNRS president when the posts were created. "FNRS has reached the maximum number of posts it can fill, limited by its budget. This year, in the University of Brussels we have no more than four posts of our own to fill; at FNRS there are three posts on the French side in all subjects. We are blocked at FNRS . . . at university . . . and we have the economic crisis."

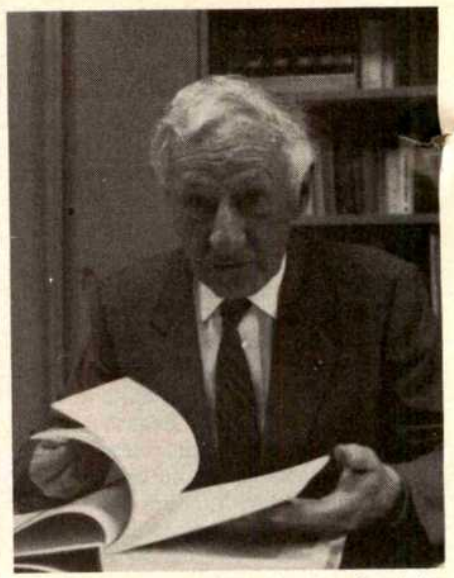
"It's very very difficult now to get a new man into research. . .", says Jaumotte.

The FNRS scheme began with ten Flemish and ten French posts, in all academic fields. Later it declined to seven Flemish and six French, because Jaumotte had imposed the sensible rule that if a university failed to give the fellowship holders a job, then they could not get any more such posts. FNRS had relied on the universities taking them up. It was not supposed to be a career position — but it has become one. In 1984 FNRS will have six Flemish and three French posts to offer — plus another two or three on the Flemish side where some universities are expected to give some existing holders posts. The uneven division between the regions is a consequence of the fact that the Flemish universities are younger and still growing.

Fifteen people who've published in *Nature, Cell* and *PNAS* competing for one post . . . it's crazy

Inevitably, with so few posts to offer, particularly on the French side, intrigue begins to play an important role in decision-making. Says Brachet "When the last FNRS director died, they didn't fill his post. So the council is now headed year after year by the rectors of the universities. There should be more independence, because finally it's the rector in charge at the time who decides."

"For example when we try to get a researcher a permanent post, we do what we can in the committees, there are fights and so on, but there are more than 20 committees, one for each field, each proposing candidates — and finally the rector in charge makes the selection in an arbitrary way. So you know that if your man has been classed first, the great thing is to work on the rector, and convince him that your man is the most excellent!"



André Jamotte, the man who introduced the "parking lot" posts.

To put rough figures on it, FNRS has a five-to-one surplus of demand over supply for research studentships; and for permanent positions ten to one. The Flemish-side administrator of FNRS considers that FNRS could double the number of research studentships without any loss in quality. "For the permanent jobs it's awful. We could take 80 per cent of the applicants."

And without posts in Belgium, some of the country's best young scientists are leaving — particularly from the French region to France, where posts and funds are much easier to come by. Said one Walloon group leader: "Two of my students are now *chef de service* at the Institute Pasteur in Paris, one is a director of the French genetic engineering company Transgène, others are in Lille, Montpelier . . . There really is a brain drain."

Even some senior people are leaving — like plant geneticist Professor Jeff Schell who was nominated director of a Max Planck institute in Cologne and now comes in to his Ghent laboratory one day a week — "this is a part-time brain drain!" said a colleague.

Another example of the problem is a researcher who as a student took a joint best first class degree in the Free University of Brussels. He was lucky enough to win a national prize for his thesis, which got him a permanent FNRS fellowship. He has been a year at the Massachusetts Institute of Technology, two years at Yale, and then came back. He might have expected a professorship — but the place he might have taken at the university, shortly to be vacated by a retiring professor, is to be abolished to save money. Anyway he would face the poor funding of science in Belgium. Now he has been asked by the French Institut Pasteur (Lille) to work in France. He would be able to take with him

In or out — a "scandal" over EMBO

BELGIUM's position in the European Molecular Biology Organization (EMBO) and the laboratory (EMBL, in Heidelberg), is somewhat confused. The story begins with the Brussels biologist Professor Jean Brachet. A few years ago he was seeking to set up a national or international biological laboratory in Belgium, on behalf of Belgian biologists. He met resistance at every level, and "that's why I was among the first to think about EMBO". Brachet played an important role in the establishment of the organization. But amazingly, Belgium is still not an official member. And Belgium does not contribute to EMBL.

"What happened", says Brachet "was that after pressure from our Nobellists Claude and De Duve the government admitted that EMBO would be a good thing, but they said that since EMBO planned to have a laboratory, and Belgium did not want a part in it, they could not join". But they pay indirectly: they give the EMBO subscription to FNRS, which pays, but the Belgian state has no official place in the organization.

This leads to a diplomatic anomaly. There should be an official representative of every EMBO state on the EMBO council, but because Belgium does not have official membership, it has no such rights. Belgium sends the general secretary of FNRS, who is allowed to listen, but not to speak — the government, it seems, fears that becoming full members would mean coughing up for EMBL.

And despite the original reluctance to establish the laboratory, Belgium *does* use EMBL. "But we are really ashamed — we do not feel comfortable", says Brachets' successor Professor Renée Thomas. "EMBO treats us very generously, in the number of people from Belgium who are elected and so on — chosen on scientific grounds alone. We have young scientists going to EMBL and they treat us as if we gave the money. It's scandalous."

EMBO costs some BF 1–2 million, and it would be another BF 25 million for EMBL. "Greece pays; Finland pays; we don't. I asked Mme Dehaux, the director of the government science policy programming office: she said listen, "we haven't even contributed to CERN (the European nuclear physics research laboratory) for two years; why do you want us to contribute to EMBL?"

The CERN payments have now been made; EMBL is still waiting. □

three people for three years, with a permanent post for himself. "It shows people have been well-trained here, at least . . .", commented a colleague.

However, a senior official of FNRS, has a more sanguine view. "It's a very tough problem" he admits. "On a first look, you would say yes, there is a brain drain, in absolute numbers. But is the final output a brain drain? I know a lot of people who emigrated to the United Kingdom or the United States, who have professorships there, who are training Belgian students who come back and enter industry here — for example in solid state, bioengineering, molecular biology. There is a brain drain, but the effects may be positive in the long term." □

Research council

Strength through division?

THE Belgian national research council for basic research, FNRS, is divided in two, almost literally from top to bottom. Downstairs in its tall Brussels block sit two women, in identical uniforms, side-by-side in identical glass boxes. Above them are different signs: one reads "reception" in French, the other in Flemish. A visitor must be sure to speak to the right receptionist: the first will connect you only to the French side of the council, the second only to the Flemish. Money is divided just as automatically: according to a politically agree formula (called a key — Belgium has many keys), some 54 per cent of FNRS funds go to the Flemish side and 46 per cent to the French.

Crazy? No, says an ex-president of FNRS, engineer Professor André Jaumotte (who is also ex-rector of the French-side Free University of Brussels), this *a priori* division keeps political, regional conflict out of scientific discussions: given the key, "there is no possible argument about the budget, so you can discuss quality without complications." The system results in "some duplications . . . it's not perfect, but it's not so catastrophic".

The Flemish-side administrator of FNRS also backs the division: because it provides independent referees. "Our boards of referees are composed of ten scientists, five French, five Flemish. They advise as one body, but in two budgets. So, say, in discussion of a Flemish project, there are five French referees who are entirely independent; and vice versa.

In fact, says the administrator optimistically, "regional divisions are not worsening the problems of Belgium — they help find the solution! I'm quite sure of that . . . ". Belgium, geographically, is the south of the north and the north of the south. So, "two mentalities meet here . . . Here, in this very small country, Anglo-Saxon and Latin attitudes stimulate each other.

However, according to a Walloon biologist, the cultural division of FNRS spreads cash too thinly. "Instead of backing a good group in one subject on one side of the country, and a good group in something else on the other, everyone is trying to do the same thing — every sum is divided." This can mean constructive competition, though says the Flemish FNRS director.

The FNRS formula of 54 per cent to Flanders and 46 per cent to Wallonia is not fixed. It is based on the numbers of Belgian graduate students in the two regions, and it shifts as those numbers shift. The trend favours Flanders. In the 18–30 age range, 65 per cent are Flemish speakers and 45 per cent French, and university attendance is increasing in Flanders, whereas it has stabilized in Wallonia. So the balance is shifting further and further towards the Flemish. It will eventually stabilize at about 60/40, the Flemish administrator believes.

But these ratios are very new. A decade ago, before the key, the French side used to dominate. "For a long while all scientists spoke French, even in Flanders, and there were few Flemish students until after the Second World War", claims particle physicist Professor Pierre Macq, dean of science at the (Walloon) university of Louvain-la-Neuve. "So the Flemish demand for grants from FNRS was low; for example even in 1970 Flanders had only 30 per cent of all physics research in Belgium, although the population was 60 per cent Flemish."

But, Macq admits "it really was an anomaly". So, at the beginning of the 1970s, it was reasonable that the Belgian premier Théo LeFévre should determine to give new funds to Flemish fundamental research, without decreasing the French side, until the Flemish and the French sides were balanced. Only half the plan worked. The government established the key, which would give Flanders its fair share. But then

FNRS — cream but no cash

THE Belgian national research council (FNRS) has always aimed to provide for the cream of Belgian scientists, mostly through peer-reviewed grants and — for the past decade — through posts to be taken up in universities. It remains the best institution to which Belgian fundamental scientists can turn for peer-reviewed funds. But FNRS simply has too little money.

And FNRS funding has declined. The budget of FNRS and its "associated funds" for medical research (FRSM), and inter-university and interdisciplinary research (FRSC) fell by 5 per cent in real terms between 1979 and 1983. Over the same period the nuclear and particle physics budget (another associated fund dubbed IISN) fell 16 per cent (excluding international subscriptions).

Contrast this with the applied research council IRSIA, which enjoyed a real-terms rise of 20 per cent, and the whole state budget which rose 37 per cent!

So why has FNRS been neglected? In these political times in Belgium, one fact stands out. Since FNRS focuses on "quality" and peer-review, politicians have no power to control its actions. Political power over other sources — such as the prime-ministerial funds (the *actions concertées*), or national programmes (such as one on energy research) can be much greater and so FNRS is forgotten. That may be the real reason why the FNRS budget has been declining steadily.

Apart from posts, FNRS also gives small research grants to individual researchers — nominally from BF 50,000 (£625) to BF 1,000,000 (£12,500) for 1 to 3 years. The very maximum is BF 1 million for one year but such sums are rare. According to Brussels biologists the sum of BF 500,000 (£6,250) for a year would be "a big grant at FNRS. . . We got BF 50,000 for three years to work on the properties of a small parvovirus . . . which is very little money . . . with no personnel".

The budget for FNRS, excluding its associated funds, is BF 858 million (£11 million) in 1984. Nearly 85 per cent of this goes to studentships and fellowships. The associated foundations have around BF 2,000 million (£25 million) to play with. The funds of FNRS proper are tied to the university budget. By the 1971 university financing law, FNRS receives a precise 4.44 per cent of the running costs (excluding salaries) of the six complete universities. "I've no definite idea what the comparable figure would be in other countries" said an FNRS official "but it must be two or three times higher."

If FNRS were lucky enough to win a budget increase, how would it spend it? "Mostly on research students" said a Flemish-side official "to increase the quality and number of PhDs, for industry as well as university life. Second, to research contracts, to stimulate inter-university and interdisciplinary work." (The Belgian universities are very monolithic, and this would help to break the barriers.) "If the R in R&D has no basic component, then forget your applied, your development. We have to go back to basic research. This is not just special pleading. You must do the research, and train people — the PhDs — who will be able to go out to industry and come back to pick up these ideas."

"We really need double the money we have now. Otherwsie I fear for the future — not just for the universities, but for the people of Belgium . . .". □

came a budget crisis; and the FNRS budget was frozen.

There was no new money to pay for the new Flemish share; so since the key had been agreed, the government could do nothing but decrease the French funding to pay for the Flemish increase. So while all Belgian scientists have had to face constant or decreasing funding since 1977, in the French part the problem has been much worse, Macq claims. "The objective was admirable" says Macq "but the result in Wallonia was disaster".

Take fundamental nuclear and particle physics, for example. They are funded through the IISN research fund (an associated fund of FNRS). IISN funding of research in Wallonia fell 20 per cent in real terms between 1977 and 1983, says Macq, whereas Flanders saw a 24 per cent increase. In research council funds other than IISN, there was a net real decrease of 11.5 per cent on the French side from 1977 to 1982, but an increase of 1 per cent on the Flemish, according to Macq's calculations.

Macq also criticizes trends in the national fund for applied research, IRSIA. "The main goal of IRSIA in 1950 was to expand research fellowships in Belgium, because the number of PhDs was very low before and after the war, except in chemistry. But between 1977 and 1982 IRSIA has increased 28 per cent in the industrial sector and 13 per cent in agriculture, yet decreased 6 per cent in fellowships."

"When you see all these numbers — you see there is something wrong in Belgium."

Biotechnology

Cooperation *can* succeed

BELGIUM is strong in basic molecular biology. It also has some strong pharmaceutical and chemicals companies. The two are getting together under the wing of IRSIA, Belgium's applied research council, in an innovative, unified scheme which breaks new ground for IRSIA, which normally puts a single company together with a single university group when dealing with universities.

Three years ago the council faced a rash of proposals from companies that were keen to start up in biotechnology. But they all wanted to associate with the same laboratories — the good laboratories. They had seen, says IRSIA advisor Dr W. Degriek, that "something was going on in the universities" and they wanted to join in. "They all needed to take the first step on the learning curve, and they counted on the universities for help."

So IRSIA called them in and told them to take the first steps together. "We said: you must all do the same first phase, and we will bring in only the best specialists from the universities — this will be cheaper and better than the individual projects you've proposed and you will all profit from it."

IRSIA did not restrict the programme to the companies that had applied, but launched a big campaign and added others. The universities then made a global proposal covering most of the companies' needs. A "biotechnology club", now with 33 member companies, was established. Members can introduce their own projects, but they have to show some "solidarity" with the ongoing, "more or less basic" research, preparing the future, training young people in that area.

"So we realized two goals in the same formula: we avoided overlap, and we created a new and larger movement", said Degriek. IRSIA has been pleased with the results and has repeated the exercise in five areas of biotechnology: in fermentation, monoclonal antibodies, genetic engineering, protein engineering, and production of methane by immobilized enzymes.

Research directions are guided by three-monthly meetings with industry, and IRSIA already has two patents out of "really high-level research."

IRSIA, excited by its success, sees this as a new system of support, which they could apply elsewhere. They call it the "common trunk" — from which branch specific company projects. "It works really well for brand new technologies, where everyone works really well for brand new technologies, where everyone still has to learn, and there is no competition yet."

Royalties from patents are to be distributed through contracts on which IRSIA claims to have done "marvellous work". The contracts are very complex, because IRSIA has had to link the interests of multinationals to those of very small Belgian companies that have completely different markets and possibilities. "You have to marry them somehow. We think that's been one of the successes, having been able to define a contract linking up that very inhomogeneous club."

IRSIA started one project in fermentation in 1981; the formula became more precise in 1982; and the last project, in protein engineering, was added at the end of 1983. There are now three "common trunks" in robotics, two in speech processing, and one in artificial intelligence.

"This is really where we really are bringing the universities in . . . it's never been so clearcut until now . . . before, either a whole industrial sector had to agree on a single project, or it was with one company and therefore rather short-term. Now all the "common trunk" meetings are very active, there's a big exchange of ideas, and the professors have really good support. They get posts, new specialists in their laboratories for a rather long period, not just one or two years."

"We really want", said Degriek "to make the building blocks for the next generation". □

Science's crisis

The logic of application

To understand Belgium one needs perspective. A source of perspective is the Belgian national council for science policy, CNPS, which has just prepared its most important document yet — a set of proposals for solving the Belgian "crisis" in science (it is not too strong to call it that) over the next ten years.

But every perspective implies a viewpoint; and some leading fundamental scientists in Belgium consider that CNPS is too beholden to industry, and too favourable to applied science, for the health of basic research. As circumstantial evidence, the complainants cite an attempt to set up a council of the "scientific aristocracy", including Nobel Prize winners Christian de Duve and Ilya Prigogine, to advise CNPS on the needs of basic scientists in Belgium. This committee of the greats was allowed to die after a two-year trial.

As a result, CNPS — and its president, Professor R. van Geen, ex-rector of the Flemish free university of Brussels and an applied physicist — has become the only effective scientific voice in or near the Belgian national government. So if CNPS were blessed, it would be significant. Moreover, CNPS wields its influence over a government already inclined to think that basic research can look after itself.

However, the accusation does not quite hold up: van Geen and his CNPS are not going to be willing scapegoats for the troubles of basic science in Belgium, although they certainly put an emphasis on application in their 10-year proposals.

So what of the composition of CNPS? With 34 members, it is designed to cover all interests in science and technology, and it must also cope with the linguistic divisions

. . . every researcher cannot do whatever he or she wants . . .

in Belgium. Those apart, CNPS represents four groups: the research establishment (rectors of universities and directors of research); the grass roots of research (young researchers in universities, government laboratories and private industry); the leaders of industry, including banks, some big companies, some small ones (the capitalists, as van Geen puts it); and trade unions and cultural groups. This broad council advises the science policy minister Philippe Maystadt ("a very clever man", who is also government budget minister).

Unfortunately, at the time of writing the CNPS recommendations on policy for the next 10 years are unpublished, unavailable and politically sensitive because, says van Geen, of the "objective" fashion in which CNPS has treated the two communities problem. But he was prepared to talk about its broad outlines.

The first difficulty with Belgian science is

money, the report says. Government spending on science was cut dramatically in 1977, and there has been no recovery. The universities were especially hard hit by the 1977 cuts — "It's the Thatcher policy...". University budgets are still decreasing. "In three of the six universities, they've stopped taking the journal *Physical Review*! Which is unthinkable!".

The proportion of gross national product spent on research in Belgium is too low — at around 1.5 per cent (0.6 per cent by government, plus another 0.9 per cent by industry). Belgium is trailing in the government sector, as a result of cuts in government spending in the face of the economic crisis. But in its 10-year report CNPS argues that Belgium needs to make an effort "precisely because of the economic crisis, not in spite of it...". The greatest difficulty in Belgium is to get this point over: "politicians often consider that research is a luxury, a dispensable item".

Minister Maystadt recognizes the problem, says van Geen, and has a plan to raise the proportion to match other countries in five years. Plans can fail, of course, and in any case the emphasis in this new initiative remains on applied research projects.

But the result of present poverty is a brain drain: "We have very good universities and scientific education — and we're a country known all over world for the quality of its scientists. It's a pity that we can't support them here...".

The CNPS report, however, does do more than cry "more!". "Every researcher cannot do whatever he or she wants" says van Geen: "research today needs more than a pencil." So CNPS suggests there should be a *selective* policy in fundamental research. The policy should be based on finding a proper equilibrium between fundamental and applied research.

The selection would focus on "centres of excellence", which van Geen defines as "those places that have the people, the equipment, and where the science is relevant". The term "relevance", of course, implies selections; but other air can enter the CNPS system, as CNPS is also aware that such centres can age and atrophy. "If you only do science in centres of excellence, at some point you will have none", as their science becomes obsolete, says van Geen. "We have that today with the nuclear fission research centre at Mol. Thirty years ago it was at the frontier of physics, now it's rather out of date."

So as well as support for the centres of excellence, there must be money — "local" budgets, van Geen calls them — to stimulate "trial, background research". For example, van Geen says that Belgium needs a centre for laser research. But if Belgium had not found the money for supporting those doing the initial work on lasers, there would be no core for a centre of excellence. So, van Geen freely admits, a management policy for basic science needs care.

Policy makers must also be prepared to wind down some centres — for example nuclear fission research — the report points out, to make room for solid state, modern optics, new materials, microelectronics and biotechnology. Scientists individually must also be flexible. "I had a policy of high flexibility as a university rector — I think it's not only possible but essential." A scientist needs mobility among fields of research; and among institutions (moving among industry, schools, universities, government); and geographical mobility.

The CNPS ten-year forward look, says van Geen, shows by example that really creative developments in science occur when some overlap occurs between disciplines. It is not sufficient to put a physicist *near* a biologist, they need to work on the same problem, the report argues. But van Geen admits some problems with a directive policy on mobility, flexibility, overlap and centres of excellence. "You must decide *who decides* about the new fields. There you must be very careful. I don't like a 'Russian' system where someone — a bureaucrat — says OK you're doing physics but next week you'll do maths and then two years later biology. You must put people in the right place; and give them also the means to be flexible, the means to learn new fields."

This, then, is the van Geen/CNPS approach to science policy in Belgium; and the reader must decide for himself or herself whether it leaves enough room for basic science to breathe. □

Regionalism

Increasing divide worries centre

THE latest, unpublished report from CNPS — the Belgian national advisory council for science policy — pulls no punches on the "community problem".

Some 55 per cent of the population are Flemish, and 45 per cent Walloon. They speak very different languages, which mark out the history of Europe: the Walloons, in the south, speak the Latin language, French, and the Flemish speak Dutch, which is close to German. The languages differ greatly, and speakers on each side have great trouble learning the other's language.

Moreover, regionalism is increasing. Nineteen-eighty was a turning point, when the national government devolved funds and powers, including policy on applied science, to new regional "executives" (so named to distinguish them from the local "governments" of a true federal system like those of Canada or Germany) plus a separate executive for bilingual Brussels.

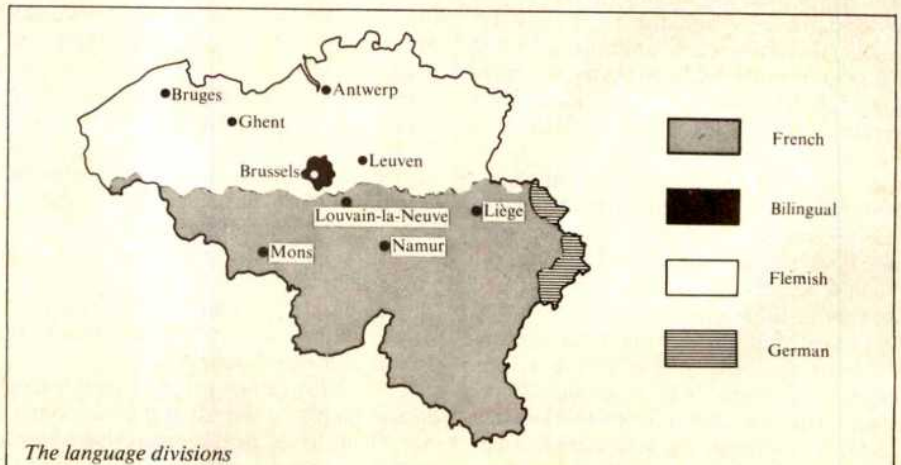
Amidst this complexity CNPS has asked the national government to clarify the funding system for science. "Science policy is very difficult now in Belgium", says CNPS president Professor R. van Geen. "Some people want a Flemish and a French science policy — but scientific research is a universal thing."

For example, take the government's science policy office (SPPS, science minister Maystadt's executive arm). SPPS is still national. But on both sides of the country there are demands that it be split in two parts.

But, pleaded van Geen, "You must be careful in what you say, as I hope the new equilibrium [between national and regional forces] will help and not hinder research".

"We try to create a balance [in the unpublished CNPS report, which advises on policy for Belgium's next ten years of science] but it is very difficult." The topic is "so electrically loaded today that you have to be very careful. Please report this carefully", said van Geen "as we don't know what the equilibrium state will be". It is "not impossible" that Belgium will split into two nations ("I don't want that — I say that clearly".) In the present circumstances, "fission" could arise out of some political or economic accident.

The community problem has flared up lately for four reasons: first, population. For a long time the French dominated both numerically and politically, but now the Flemish have become numerically dominant and are demanding a redistri-



The language divisions

bution of money, industry, jobs and political power. Today the Walloons feel they are the victims, when for a century the reverse was true.

Second, Belgium is going through an economic crisis. In the past, community problems have been solved by throwing money at them, yielding equally to "the four forces": Flemish, French, Catholic, and non-Catholic (which means agnostic, rather than protestant, in Belgium). But now Belgium has to decide where to put the little money it can spare: it cannot afford equilibrium.

Third, Flanders is undergoing a rapid economic expansion, with green-field sites for new industry and a more docile population (in terms of strike record) than Wallonia, weighed down by big, troubled industries like coal and steel.

And fourth, regionalism is increasing everywhere in Europe: the Basques, Brittany, Scotland for example.

So far there has been no serious violence over the regional issue in Belgium — but it could happen. Belgians look in fear at Northern Ireland. And there is a possible flashpoint: a small community of 4,000 people in Belgium which is contested between both sides: a small bit of land which has become a symbol. To judge by some newspapers, the major political problem in Belgium today is that the mayor of this community cannot speak Flemish. There has been some violence there — guns, bombs — but so far without victims. "Violence is increasing", says van Geen, "I just hope it will not explode." □

New technology

Flanders sets the pace

FULL of bounce, the Flanders regional executive, in just its third year of operation, is leaping into high technology.

High technology is political: the president of the executive, Mr Gaston Geens, an economist by training, has grasped technology as the lever that will lift Flanders above the rest of Belgium and establish it as a state within a state. And it is all happening very swiftly, after the law of August 1980 that regionalized a measure of responsibility for economic policy and applied research. (Fundamental research and the basic funding of the universities remain national.)

The Wallonian executive has seen the challenge set by the breathless Flemish executive, and is also making strong efforts — particularly in biotechnology — but it has the harder task. Wallonia has the old industry, Flanders the new, and moreover Flanders is going through a kind of spiritual rebirth, emerging from a century and a half of French domination.

It may be, of course, that Flanders will pull the whole of Belgium with it, and the regional competition will prove highly creative; but meanwhile everyone in Flanders knows what high technology means for the region and its politics — independence — and there is almost no public or trade union opposition to the changes, even though, for example, rampant robotization of Flanders factories is creating no new jobs and eliminating some old ones.

Flanders did have some old industry, mainly in textiles, and that has suffered in the general recession like Wallonia's steel and coal. But, claim Flanders officials, the Flemish textiles industry is now automated and competitive again.

The officials also say that Flanders now accounts for 70 per cent of all Belgian exports, compared with Wallonia's 25 per cent. (The rest comes from the Brussels region.) Moreover, last year there was a 21 per cent increase in investment in Flanders, says the executive, whereas the total investment in Belgium is decreasing.

Ford, for example, is investing some BF 10,000 million (£125 million) to automate its Flanders factories. Employment levels will be unchanged, but, says the executive, if it did not invest, employment would fall with competitivity.

"The people accept this better here than in Wallonia", says Luc van Nuffel, microelectronics adviser to Geens. Recently, there was a one-day general strike in Belgium, in protest at the economic crisis. The strike was better observed in the French region than the Flemish, and — claims van Nuffel — if the unions were not national, there would have been no strike in Flanders at all.

It is against this kind of background that the Flemish executive is backing the new technologies — and it has been able to move fast by making use of the many reports of the national advisory council on science policy (CNPS). The executive has moved nowhere more rapidly than in microelectronics. In this, and other developments (the executive has three programmes, on new materials and biotechnology as well) the executive is attempting to move in harmony with the three universities of the region: the Catholic University of Leuven, the Flemish Free University of Brussels, and Ghent.

However, the executive believes in concentration of its resources (which total 7 per cent of the whole national government budget) and so it is proving a force for selection, which has suffered in Belgium because of the usual balance that had to be struck between the "four forces". In microelectronics, for example, the executive focused on Leuven, but in such a way as to draw in the other universities in an inter-university scheme.

The Flanders microelectronics education plan is almost literally exponential. Flemish electrical engineers have been, and will be trained in 14 industrial (technical) colleges and the three universities. "The problem was that only one university [Leuven] was training the people that industry needed", said van Nuffel. To transfer this knowhow the excecutive took four lecturers from outside Leuven and put them through a very intensive, specially-designed course at Leuven in very-large-scale integrated circuit (VLSI) design. The following year these four trained another thirty. In the third year (which begins this September), these thirty will go back to their institutions (universities and technical colleges) and introduce courses on VLSI.

Moreover, in order to intensify the training, the executive has set up a computer network based in Leuven but linked to every teaching institution. Students in colleges throughout Flanders will use it for real-time, computer-aided chip design, using the Leuven software and equipment.

The expected result: that every year from 1986 onwards Flanders will be producing 200–300 engineers with specialist experience of custom VLSI design. VLSI research will be concentrated in a new BF 2,300 million inter-university centre at Leuven: IMEC. A company (Inventive Systems) has been established to promote chips in industry. And Flanders has given 49 per cent backing to Bell-Antwerp (an offshoot of ITT) to set up a custom-chip production plant at a total cost of BF 3,000 million. The plant is to be operational in 1985, and will have the capacity to make an estimated 4 per cent of the needs of Europe. Around 40 per cent of the production will go to ITT, 60 per cent for outside sale. The objective in backing Bell-Antwerp was to give Belgium some strategic independence in the chip market, but van Nuffel could not estimate what fraction of Belgian demand the factory might satisfy.

The executive has worked out similar programmes for materials and biotechnology, but these are still at the planning stage. In materials there is a study concerning the setting up of a laser applications centre; and in biotechnology the executive was expecting the results of a study on a communal, time-shared pilot plant in May. (The plant would be either in Brussels or Ghent.)

Eventually, the regional executives hope to get more money to play with, as certain national laboratories and funds are regionalized (often against their will). For example the national applied research council IRSIA had just been officially one-third regionalized: a third of its budget divided between Flanders and Wallonia. Flanders will get 60 per cent of that one third, and IRSIA's advice on the distribution of that fraction in Flanders will be advice to president Geens rather than to the national government. Says van Nuffel: "it makes no sense, say, if IRSIA supports traditional industry while we support new technology". Thus IRSIA and other institutions will increasingly feel the pressure of regional politics. □

Microelectronics

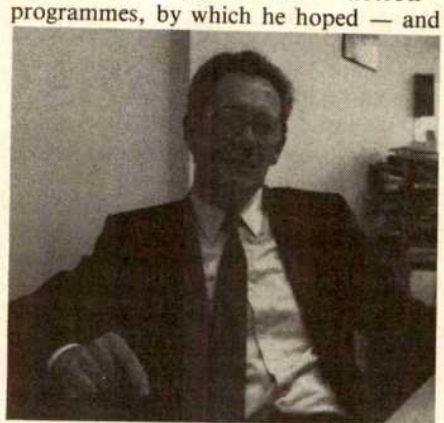
Silicon Valley comes to Belgium

PROFESSOR Roger J. van Overstraeten speaks with hardly a pause, and with constant intensity: it is easy to see how he has established — in less than 20 years after graduating from the University of Stanford, California — an extraordinary microelectronics laboratory in his native Belgium, a laboratory which now almost flies free on industrial contracts, depending on the government for only 20 per cent of its budget.

However, that proportion is about to change: the Flemish regional government is investing capital of BF 2,300 million (£30 million) and annual expenditure of BF 1,000 million (£12 million) to expand Overstraeten's group into an inter-Flemish-university laboratory: IMEC (the inter-university microelectronics centre). Reflecting general amazement at such expenditure in the straitened circumstances of Belgium, IMEC has earned the title: "Superlab".

The nucleus of IMEC will be van Overstraeten's group ESAT (electronics, systems, automation and technology) which he started in 1965 as part of the electrical engineering department of the (Flemish) Catholic University of Leuven. ESAT is highly renowned in its field. According to the research vice-president of the Dutch-based multinational Philips, ESAT's work on computer-aided design of chips puts it second only to Berkeley, California. ESAT has already spawned a clutch of successful small companies in the United States and Europe, and is highly sought-after for consultancy work.

ESAT started slowly, with a staff of just eight people in 1970. "That's when we really got a start. We had proved that it was possible to do some original work in microelectronics in Belgium. . .", and as a result the group won one of the first handouts from Prime Minister Théo Lefévre's "concerted action" programmes, by which he hoped — and succeeded — to improve the financial and competitive position of some of Belgium's top groups.



Van Overstraeten — boss at "Superlab"

As say many of Belgium's researchers, the concerted action cash was extremely important. At ESAT, the improvement was something like an order of magnitude. By 1975 ESAT had 60 people, when it merged with another laboratory to reach 85. Its present 150 is "saturation" for the space and facilities available — hence Superlab.

ESAT works in three areas. The most important, "integrated systems", deals with everything to do with integrated circuits, including ESAT's strong group in computer-aided design (which started early, in 1969), and a group dealing with the physics and chemistry of chip manufacture.

"We have developed 5 micron CMOS technology, 3 micron CMOS technology, and transferred it to industry. It's unique that a university laboratory should go so far [towards industry] says van Overstraeten. "But we did it partly because there was no Belgian company in the field — we did it to prove what Belgium can do". Now there are five Belgian chip companies, some of them headed by ESAT staff.

Van Overstraeten's ambition is national: why else would he have come back from Silicon Valley? And he set up in university rather than industry "because I think that education and training are extremely important — and training is still our main objective. Every year at ESAT we train 100 electronic engineers in this field. [Superlab will double or treble that number.] And all of them have the chance of designing integrated circuits."

"So I think that a laboratory like this should be between university and industry. It's not a typical university laboratory, but it's not an industry laboratory either."

Belgium will also soon have MIETEC, a joint venture between Bell-Antwerp and the Flemish executive, due to begin producing custom chips in October 1985. "I don't think a company like that could have started in Belgium without the people we have trained."

ESAT also works in systems and signal processing. More and more this field will require custom-designed integrated circuits, van Overstraeten believes. Part of the signals research is fundamental mathematical work in cryptography. For the implementation of a code is always a specific integrated circuit, "and more and more it gets difficult to separate these things . . . a mathematician alone can end up with something that would be difficult to implement in 20 years," whereas codes are needed on a 5–10 year time scale.

The third area of ESAT work is photovoltaics — based on technology similar to that of integrated circuits. ESAT developed a screen printing technology for making solar cells, 4 years ago, as an application of existing integrated circuit technology. The process is materials-efficient, and is now the property of a new Belgian company, Photon Technology, doing well according to van Overstraeten. . .

There are other laboratories like ESAT on a scientific level, van Overstraeten admits. "But if I take integration with industry, there's none like it. It's different from Stanford, and from Berkeley. There the industry is to hand, here we had none." And ESAT has international links with industry in the United States, Switzerland, Germany, Holland, France, the United

Professor van Montagu's local industry

MANY Belgian basic scientists are now turning to applied science — but some with more success than others. One of the more successful ones is Professor Marc van Montagu, plant geneticist at Ghent. Van Montagu has contracts with many companies, but has also set up his own — just started last year: Plant Genetic Systems (PGS). (Van Montagu's Ghent colleague, Professor Walter Fiers, also runs a company — an offshoot from Biogen, a genetic engineering company: Bioghent.)

Such enterprise is not unknown among molecular biologists — but van Montagu's PGS does stand out for one unusual policy: "We mostly hired people locally — in Belgium. We've seen other groups take people from all over the world, but they just end up with a collection of competent but competitive people, without a common spirit. So we wanted to take a different approach, to get the spirit going first, to show we can do it, and then give the team additional help by injecting foreign people if it needs them."

PGS will "apply our techniques to engineer plants to improve their commercial value, for example for resistance to viral and fungal disease, to increase nutritive value. Those are the things we think we can do now . . . and also a lot of work on soil microorganisms. And we have started in association with the university of Brussels (ULB) a programme on protein engineering."

The students don't object to working with industry, says a "pleased but surprised" van Montagu. "Most of the younger students find it challenging to do applied research . . . I used to think that they would object for political, ecological, or other reasons." They don't.

His group has a patent on some Ti plasmid vectors "but we decided the money should go to the university. We don't know if it's making money yet. It takes a long time."

PGS was started with BF 400 million (£5 million) and must live from the interest on that capital plus contact research. The goal for the end of 1984: to be in balance on the current account. □

Kingdom — "that's quite unique too" claims ESAT's director.

But ESAT still needs government support: "Our funding now is quite strange, we are living mainly on projects — only 20 per cent comes from the university and fixed funding — that makes life very dangerous" — particularly when electron beam apparatus can cost $3 million.

ESAT has been supported by three concerted action programmes, by national programmes, by the EEC, IRSIA, the science policy programming service, FNRS, and several ministries. The university pays 25 salaries. "But most important is that the university allows us this openness, and that they support us in getting funds." Van Overstraeten says he is not bothered by the lack of university posts. He can make do on soft money. And the people who work in his laboratory are sure of a job in industry.

And now comes Superlab, IMEC: it will be an important laboratory, with 150 permanent staff and 100 people working on projects in design and technology. (ESAT's present budget is about a third of IMEC's projected spend.) IMEC, available to the universities of Leuven, Brussels (Flemish side) and Ghent, and technical colleges, will also have closed-circuit television, so the courses and seminars at IMEC can be followed at the other centres, with audio feedback for questions. "It's a new formula for a research laboratory, an inter-university centre combined with interaction with industry".

IMEC could not have been founded without government investment. The investment in people is so huge, a university could not do it. But the *national* government could not have begun IMEC — because it required a choice between regions and a focusing of funds.

. . . Belgium may sound complicated. . . but finally things seem to work out quite well. . .

IMEC is thus "a Flemish thing, but the research will be European — and we'll work for French-speaking companies, there's no problem there". Nevertheless, says van Overstraeten, "If I look at Maystadt [the national science minister] he's doing excellent things. . . Belgium may sound complicated and it is, but finally it seems to me things work out quite well. . . ".

Flanders is developing fast, the mentality is different, the industry is different, it is a challenge for the French side, but, says van Overstraeten, "I don't think they'll stay behind". □

Luxembourg

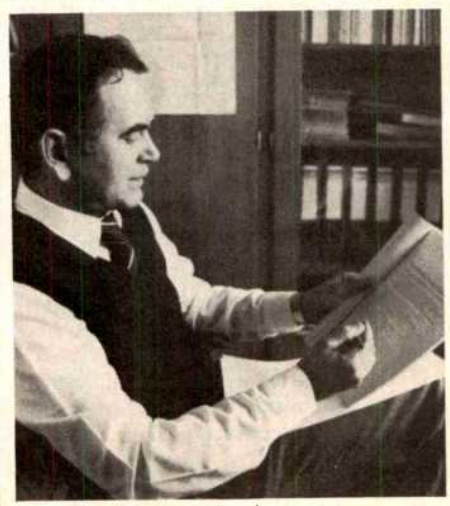
Old industries in decline

RESEARCH in Luxembourg? It does exists. Luxembourg boasts a long-dead Nobel Prize winner and in 1977 the Grand-Duchy began to expand its tiny research capacity. But Luxembourg's government science is on a very small scale — just 12 researchers in the basic sciences, who really are only nominally independent: as they recognize themselves, they depend absolutely on collaboration with groups abroad. Perhaps only 0.1 per cent of the Luxembourg gross national product is spent on both basic and applied research (but there are no official figures).

The Grand-Duchy has only the population of a large London borough — 366,000 people — and no full university, although it has 3,000 in university education, mostly abroad. But it had the coal and iron ore to justify a major steel industry (Arbed), which ten years ago employed half the working population (now the fraction is plummeting to a quarter). Arbed has a research laboratory with 30–40 staff. Luxembourg also hosts the Goodyear tyre company's European research laboratory (with some 150 researchers). And some smaller companies also do research in Luxembourg, mostly on metals and materials.

But Luxembourg really could do more research, and needs to in order to be prepared for the new industries that must follow steel. And, indeed, in 1977 a Mr Robert Krieps, minister for cultural and scientific affairs in the — rare in Luxembourg — socialist coalition government, began a slight movement in that direction. Krieps created a number of new institutions for research, among them an advisory council for research policy headed by chemist Professor Pierre Seck. One of the biggest new creations, says Seck, was a small-scale teaching hospital where some important research is done. And Krieps created institutes for technology (technical colleges) that do some applied research.

But despite 3,000 potential students, Luxembourg does not have a complete university. What it does have is the beginnings of one: the Centre Universitaire (CU) with 800 students, created ten years ago, which offers just the first year of university studies. (This is where Luxembourg does its basic research.) The CU first year is intensive, with an amazing student-teacher ratio of 2 to 1, and CU courses are widely recognized by other universities in Austria, West Germany, Belgium and France.



Pierre Seck — not quite alone.

If CU staff, like Seck and his five colleagues in the department of science, want to do research "we have to collaborate with a team abroad". CU research interests can be listed in total: a collaboration with a French CNRS director working on ecty-steroids in insects at the University of Strasbourg has revealed the same ectysteroids in worms; chemistry at the CU has focused on heterocyclic and organometallic compounds, in collaboration with the universities of Metz, Heidelberg and Louvain; the CU physicist works with Centre Hospitalier on medical electronic signals, seeking correlations with disease. And there is one pure mathematician, in a world of his own as far as Seck is concerned. . .

The CU department of science has around 40 teachers, but only Seck and his five colleagues are employed by the CU alone: 20 of the others are upper-level teachers at *lycée*, qualified to teach both at secondary and tertiary level. Then 6 come from foreign universities on secondment, and 10 are professionals such as doctors and engineers.

Research in the CU is done by the 6 full-timers. "But we've recently won a new freedom — to release some of the secondary school teachers for several days a week." That gives a total of about 12 man-years per year of research capacity at the CU, except for the teaching load.

It is small team, but, says Seck, his laboratories "are very well-equipped for the subjects we've chosen" with apparatus like a Mossbauer spectrometer, NMR and X-ray diffractometers, and the department is promised a new building.

Thus basic research in Luxembourg proves viable, at least while it remains linked to the science going on in surrounding countries, and while it is not so ambitious as to outstrip the country's capacity.

The only trouble is that Luxembourg science is like an unfinished painting, a few dabs of industry, public and private research that could do with a little coordination. "We have to grow together." Aware of this, in 1983 the members of the research advisory council demanded more power to coordinate and fund Luxembourg's science. The council would not continue without that power, the members threatened.

But the government — since 1979 led by the Christian Socialist (right wing) party that has ruled Luxembourg almost continuously since the nineteenth century — called the bluff and simply failed to renew the council's mandate. At the time of writing the council's future is unclear, but Seck has hopes for new developments after the June national elections. □

Fourier's law obeyed — official

Analysis of a mechanical model characterized by deterministic randomness (chaos) allows verification of elementary principles of heat conduction. But it may have other value.

THOSE who hold that the reductionist programme is a mistaken attempt to account for all phenomena in terms of elementary processes usually look for evidence to support their case in the behaviour of living things. They may be well advised, it seems, to pay closer attention to a long-familiar part of classical physics and in particular to the theory of heat conduction.

The problem, as a little reflection will show, is that there is still no unambiguously unobjectionable way of calculating from the laws of dynamics as applied to atoms and molecules the most elementary principle underlying the phenomenon of heat conduction — the notion that the rate of heat conduction between two parallel plane surfaces in some medium is proportional to the temperature difference between them, widely known as Fourier's law. From this simple statement follow the differential equations which specify the time-dependent variation of temperature or of heat flux in conducting objects of all kinds. Calculations based on Fourier's law have naturally been widely used in tasks as different as the inference of temperature distribution within the Earth and the design of industrial heat exchangers. So in what sense can Fourier's law be less than well grounded in physical principles?

The familiarity of Fourier's law no doubt blinds us to its non-trivial character. It is not, however, difficult to construct other plausible phenomenological schemes to describe the rate of heat conduction through material objects. The most obvious modification is that suggested by the process of radiative heat transfer: each element in a body at a temperature different from absolute zero might give rise to an isotropic heat flux which is simply a function of the temperature of the element. For small temperature differences, Fourier's law would be approximately true, and while the rate of heat conduction through a fluid from some solid object would awkwardly be a function of the temperature of that object, it might in practice be hard to distinguish such a variation of conduction away from a source of heat from the variation with temperature of the heat conductivity of media of all kinds.

But surely, even schoolboys will ask, has not this issue long since been dealt with? Heat conduction through gases is familiarly dealt with by kinetic theory, with results entirely in accordance with Fourier's law. For more general fluids, it may be necessary to take account of more complicated treatments of transport in kinetic systems along the lines originally proposed by Enskogg, but the objectives are the same — to calculate the rate of transport of kinetic energy from the distribution of the momenta of particles and the chance of collisions between them. In metals, on the other hand, it is more prudent to start from the likely excitation of conduction electrons into states of higher energy, and to allow for the interruption of free electronic motion in such a state by the vibration of the underlying lattice. Although most of this is intricate, in principle it is straightforward. So why should Peierls (in 1961) have written that the derivation of Fourier's law from elementary dynamics is "one of the outstanding unsolved problems of modern physics"?

Peierls' remark derives from his own work in the 1950s on the interaction between lattice electrons and the spacings of the underlying lattice. That the interaction may sometimes mean that a regular lattice breaks up into consecutive insulating patches or, alternatively, will permit the efficient transport of electrons in the forms of "solitons" is now part of the familiar understanding of conduction in solids, superconductivity in particular. The deficiencies of the theoretical foundation of Fourier's law consist, from this point of view, in the way in which all attempts to calculate heat transport (and other transport processes) from first principles rely, at some level, on an averaging process.

This is the starting point for a calculation published by Joseph Ford and Franco Vivaldia (Georgia Institute of Technology), Giulio Casati (Milan) and William M. Visscher (Los Alamos) at the end of last month (*Phys. Rev. Lett.* **52**, 1861; 1984). Their first task is the choice of a deterministically random (or chaotic) mechanical system with which to model a solid heat conductor. Such a system must be simple enough to be calculable, at least numerically, yet free from exceptional modes of behaviour that might vitiate the modelling of thermal conductivity and yet sufficiently complicated to be vaguely reminiscent of the real world. With fashionable but needless whimsy, the authors settle for what they call a "ding-a-ling" system — a one-dimensional array of hard spheres similarly anchored by symmetrical restoring forces to fixed lattice points with equal freely moving spheres sandwiched between them. At the end of the array are two thermal reservoirs, which communicate with the conducting array by means of similar freely moving spheres, which if accepted into the reservoir are promptly returned with a velocity specified by the velocity-distribution appropriate to the corresponding temperature.

The model works straightforwardly. The stiffness of the lattice-bound particles has to be great enough for chaotic motion to supervene. What happens is that the oscillating spheres act as relatively unresponsive transducers for energy carried by the intermediate freely moving spheres. The rate of energy transport can be estimated from the rate at which energy emerges from the high temperature end of the system, or from the equal rate at which it arrives at the other. The numerical calculations have been carried through fully only with very short linear chains, with two and four anchored spheres respectively. The result, cheerfully, is a constant coefficient of thermal conductivity (within ten per cent or so). The argument has, however, been made more plausible by means of a calculation of next-nearest neighbour correlations (between neighbouring anchored spheres) for a more general system, from which again a constant thermal conductivity is derived.

So far, then, so good. Here is a simple dynamical system with many of the recognizable characteristics of a heat-conducting solid which does indeed transduce energy in accordance with Fourier's law. Many people will no doubt now be tempted to embark on generalizations of this simple model. Two dimensions? Even three dimensions? Excessive zeal of that kind would be misplaced. What Casati *et al.* have done is to demonstrate that a dynamical system does indeed behave as would be expected under the influence of a random input of impulse. In passing, they have also been able to show that in strictly mechanical systems such as these, the exceptional dynamical solutions, the putative solitons, are no great encumbrance if the vibrational frequency of the anchored spheres is sufficiently great. The other side of this coin is that such models might indeed be used for studying energy transport in, say, superconducting materials. Whether reductionists should be alarmed by all this is quite a different matter, although some of them may be dismayed that this may be that hitherto elusive problem for which only computer solutions are attainable.

John Maddox

Palaeontology

Biased record of early life

from Malcolm Walter

IF an expedition to Earth from some other planet were to be mounted, and if the budget restrictions were such that only two samples could be collected to determine the history of life on Earth, it is probable that the palaeontological advisors to the expedition would urge the collection of a black chert to represent the first few billion years of biological history. Suppose, then, that the collectors travelled first to an island in Hudson Bay, where they gathered a sample of the 1.8×10^9 year old black chert[1], and then to Shark Bay in Australia or some similar arid shore[2], where they gathered a sample of extant life from the intertidal zone. When the two samples were analysed on return from Earth, it would be concluded that there had been no evolutionary progress for at least 1.8×10^9 years, and that the Earth was still in 'the age of cyanobacteria'.

Palaeontologists are aware of biases resulting from selective preservation. Disproportionate attention to cherts is not a result of naiveite on the part of the palaeontologists who work on Proterozoic sequences, but is a consequence of the very success of the studies concentrated on cherts[3]. Ever since the seminal discoveries in the 2×10^9 year old gunflint chert of Ontario[4], black cherts have been known as a good preservative for microbial cells, and consequently many studies, including that of Zhang Yun[5] on page 547 of this issue, have focused on them. As a result, our view of the Proterozoic history of life is probably grossly distorted. Zhang Yun adds to the small but steadily growing list of described microfossil assemblages which represent much of our information about life in Early Proterozoic times (1.65–2.50×10^9 years ago). Although there are techniques, such as the study of stromatolites and isotope geochemistry, that give us different sources of information about ancient life[1], microfossils are preserved cells and the most direct evidence we have. Of the various sources of information available to palaeontologists, microfossils have received by far the most attention. They are preserved in very finely crystalline silica, chert, and as a result of circumstances not yet well understood, most Proterozoic microfossiliferous cherts appear to have formed in the marine intertidal environment.

The attention to a limited set of palaeoenvironments has consequences in biostratigraphy as well as palaeobiology. Any attempt to use chert microfossils in biostratigraphy is greatly limited even before it begins, unless we can find cherts that preserve samples of microbial life from more than just the intertidal zone. Like a few other assemblages, Zhang Yun's could represent a microbiota that was unique to the Early Proterozoic. The first reaction to this new report is to view it as an encouragement to biostratigraphers, but caution is needed. As in the case of most of the other examples, including the original one from the famous Gunflint Iron Formation, the Chinese example comes from rocks whose depositional environment has not been determined by modern sedimentological techniques. The fact that its microbiota are distinctive may therefore signify nothing more than that it comes from a distinctive palaeo-environment (Zhang Yun suggests the shallow sea in a region of volcanic activity) that has, so far, only been sampled in the Lower Proterozoic — a familiar problem to palaeontologists.

Proterozoic microfossils are also found in shales, where they are called acritarchs, but there they are studied by different techniques and usually by different scientists from those who work on cherts[6,7]. Many of the shales were deposited in offshore marine environments and, as a result, contain a sampling of the marine plankton of the time. They can be expected to have been more diverse than the intertidal microbiota, not having been limited by the environmental extremes that characterize the intertidal zone. Shales should therefore provide a fuller record of evolution through the Proterozoic, and the material should provide the basis for a successful Proterozoic biostratigraphy. A combination of the palaeobiological approach that has been applied to cherts and the empirical biostratigraphical approach of the acritarch workers will give us a true picture of life in the Proterozoic, and provide the basis for sound biostratigraphy. Such work has begun[8]. □

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Oncogenetics

Progress in malignancy

from Miranda Robertson

TWO rare tumours of childhood are proving to be a remarkable source of insight into the genetic steps by which cells may progress in malignancy. The tumours, retinoblastoma and neuroblastoma, have two significant features in common. First, they are both derived from embryonic precursors of nerve cells, which normally lose the ability to divide once they have differentiated; and second, both may be associated with chromosomal deletions. These two facts have been incorporated in the following hypothetical schema. The chromosomal deletion contains a gene that is normally responsible for turning off the embryonic phenotype, including the ability to divide; in its absence, embryonic genes remain active and the proliferative capacity of the cell is retained. It has been suggested that some of the cellular proto-oncogenes may be genes that are normally active only during embryogenesis; and with this in mind, Lee *et al.* have looked for an active oncogene in cells from ten fresh retinoblastomas. They find that the N-*myc* gene is transcriptionally active in all of them and amplified in two[1]. At the same time, Schwab *et al.*, who originally discovered the N-*myc* gene[2], report that in retinoblastomas N-*myc* amplification is associated with late stages of tumour progression[3], and thus may have prognostic implications.

N-*myc* is an oncogene by an unusually complex chain of inference. It was first detected by Schwab *et al.* in the amplified DNA of a number of neuroblastoma cell lines through its limited homology to the c-*myc* cellular proto-oncogene. Thus its identification as an oncogene rests on its relationship to a known oncogene and its presence in DNA that is amplified in tumour cells. Its activation in a second neurectodermal tumour — retinoblastoma — strengthens the evidence implicating it in neural tumorigenesis.

The first step in the development of neuroblastoma and retinoblastoma must occur before the differentiation of the central nervous system is complete, and indeed may be inherited. The hereditary cases are often associated with chromosomal abnormalities involving the deletion of material from chromosome 1 in the case of neuroblastoma[4], and from chromosome 13 in the case of retinoblastoma[5]. This is believed to predispose individuals to develop the tumours, but does not make it inevitable, presumably because a normal allele of the deleted gene remains on the intact chromosome. In the case of retinoblastoma, there is now very good evidence that the second step in tumorigenesis is the inactivation of the normal allele[6]. (The mechanisms by which this may occur in retinoblastoma and other childhood

tumours have been the subject of two recent articles in *News and Views*[7,8].) Whether the inactivation of both alleles is sufficient for malignancy is not known; but there is some evidence for activation of a member of the c-*ras* oncogene family in a neuroblastoma cell line[9], suggesting that it may not be.

What is the significance of the transcriptional activation and amplification of N-*myc* in retinoblastoma and neuroblastoma cells? Nothing is known about the function of the N-*myc* product, but c-*myc*, with which it has some homology in the 5′ coding region, is implicated in the control of proliferation in several different cell types[10], and it is possible that N-*myc*, whose expression seems to be confined to cells of neurectodermal origin[11], has an analogous role in neural cells. In neuroblastoma moreover, the level of transcriptional activity seems to be correlated with the degree of differentiation of the tumour cells[11], which can vary considerably within a single tumour. Schwab *et al.* have shown by hybridization *in situ* that N-*myc* expression is higher in the less differentiated cells.

The association of N-*myc* amplification with tumour progression is suggested by investigations by Brodeur *et al.* on a series of 63 tumours taken from untreated patients at four different stages of the disease, defined chiefly by the extent of dissemination of the tumour cells. Brodeur *et al.* find no amplification of N-*myc* in 15 tumours at stages 1 and 2; but amplification in half of the 48 tumours from stage 3 or 4 patients[3]. The prognosis for patients at these later stages is usually extremely poor, two-year survival being 10–30 per cent, compared with 75–90 per cent for earlier stages. If the correlation with N-*myc* amplification holds up, it will be important to discover whether the absence or presence of the amplified gene distinguishes late-stage patients who may survive from those who will not. Such a prognostic tool could help with difficult decisions on the more heroic forms of therapy in advanced disease.

More fundamentally, further research on the interactions of the several genes now implicated in the genesis of neuroblastoma should help to illuminate the mechanism of neural differentiation and its disruption in tumorigenesis. □

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Particle physics

How neutral are atoms?

from Frank Close

UNIONIZED atoms are electrically neutral because the negative charges on the electrons precisely balance the positive charges on the nuclear protons. This concept of balancing is so deep-rooted in our scientific culture and so naturally presented in school classes that we hardly give it a second thought. In fact it is really rather profound; why should electrons and protons, which seem to be independent forms of matter, know so precisely the magnitude of each other's electrical charge?

An answer that has suggested itself to many is that ultimately there is but one type of matter: electrons and protons are siblings. If so, this would be the culmination of a long search for an underlying unity in nature. At the beginning of the century scores of atomic elements were thought to be the templates of nature's infinite variety. Then it was realized that three 'elementary particles' — electron, proton and neutron — were the common building blocks of all atoms. Later the proton and neutron were discovered to be built from more basic particles, called quarks. Now we believe that quarks and leptons are the two fundamental families of matter. Yet the precise identity of their electrical charges hints that they might not be totally unrelated, and in the grand unified theories (GUTs) uniting the electromagnetic, weak and strong forces, one finds that quarks and leptons are siblings. This startling result is a consequence of the electrical charge equality between the electron and proton. But how sure are we that these charges are identical, and what consequences would ensue if they were different?

Einstein had suggested that the electron and proton charges (Q_e, Q_p) might not be precisely equated and that:

$$Q = (Q_e + Q_p)/Q_p \sim m_p/m_{pl} \sim 10^{-19}$$

where m_p and m_{pl} are the proton mass of 1 GeV and the Planck mass of 10^{19} GeV respectively. Twenty-five years ago Bondi and Lyttleton noted that if $Q \sim 10^{18}$, electromagnetic repulsion would dominate gravitational attractions among hydrogen atoms on a galactic scale and the expansion of the Universe could thus be explained within Newtonian mechanics (*Proc. R. Soc.* **A252**, 313). Since that time the hot big bang model has been established so we may now take this as a possible cosmological bound on $Q < 10^{-18}$ for hydrogen.

The first precision test on the electrical neutrality of atoms was performed by A. Picard and E. Kessler 60 years ago (*Archs Sci. phys. nat.* **7**, 340). In their experiment, all ions were removed from a vessel containing a gas. As the gas leaked out of the vessel, they measured the change in the vessel's electrostatic field. If $Q \equiv 0$ there should be no change. Their limit for Q was $< 2.2 \times 10^{-19}$ for carbon dioxide. Other experimenters have used this technique on nitrogen and argon and have typically obtained limits $< 5 \times 10^{-20}$, consistent with the standard folklore value of 'zero' but perhaps not a definitive falsification of Einstein's hypothesis. Indeed J.G. King applied the Picard–Kessler technique to hydrogen and helium and obtained a small negative charge for hydrogen of $(-2.5 \pm 1.5) \times 10^{-20}$ and a small positive charge for helium of $(4 \pm 2) \times 10^{-20}$ (*Phys. Rev. Lett.* **5**, 562; 1960). Subsequently, King has claimed, but not to my knowledge published, details of a null result for H_2 and for He where $Q < 6 \times 10^{-21}$.

What about elementary particles that are believed to be uncharged? A nonzero charge for the neutron can be sought by passing neutrons through an electrostatic field. The most precise limit appears to be due to R. Gahler *et al.* (*Phys. Rev.* **D25**, 2887; 1982): $Q(n) = (-1.5 \pm 2.2) \times 10^{-20}$. For the neutrino, the value comes from astrophysical arguments. If neutrinos are electrically charged, then photons can convert to neutrino–antineutrino pairs and affect the rate of stellar evolution. We know that our Sun has survived for over 5×10^9 years and this places an upper limit on the rate of energy loss by a possible neutrino pair production. J. Bernstein *et al.* find that this limits the neutrino charge: $Q(\nu) < 10^{-13}$ (*Phys. Rev.* **B2**, 1227; 1963).

If, as generally believed, electromagnetic interactions are described by a local and causal field theory, then electric charge must be conserved. Thus in the β-decay of the neutron into proton, electron and neutrino, $Q_e + Q_p = Q_n + Q_\nu$. So if one accepts charge conservation, it provides a better limit on Q_ν than the astrophysical argument. But there is nothing that requires each side of this relation to equal zero.

The question of the electrical charges of fundamental particles has recently been investigated by Okun *et al.* of the Institute of Theoretical and Experimental Physics, Moscow (*Phys. Lett.* **138B**, 115; 1984). In electrodynamics, and in the electroweak theory of Glashow, Salam and Weinberg, the electric charges are not fixed: proton and electron could have arbitrary charges. The reason for this lies in the mathematical group structure of the theories. Electrodynamics is based on the gauge group $U(1)$, and the electroweak theory on $SU(2) \times U(1)$. The $U(1)$ factor gives a freedom to the charges of the matter particles which form the representations of the group. The β-decay equality must be preserved, but the magnitude of $Q_e + Q_p$ is arbitrary. However, in GUTs based on compact semi-simple groups, such as $SU(5)$, the electrical

charges are determined. If the electrical charge operator is a generator of a semi-simple group, then the sum of the electrical charges of the particles in a multiplet of the group vanishes. In the simplest GUT based on $SU(5)$, the fundamental quarks and leptons form two distinct multiplets and hence two separate combinations of charges vanish. Together with the β-decay constraint, this places sufficient constraints to determine the charges of all the quarks and leptons. The electron and proton charges are identical and of opposite sign; the neutron and neutrino charges are both zero.

Leptons and quarks belong to the same multiplet and so group transformations can convert quarks into leptons. As a result, protons, made from quarks, can decay. Searches for proton decay are in progress; the candidate events and lifetime do not appear to be in accord with the simplest $SU(5)$ GUT. GUTs based on other gauge groups are still viable. The gauge group $SO(10)$ is among the best investigated. Here the quarks and leptons fit into a single multiplet and hence only a single set of charges vanishes: the charges are less constrained than in $SU(5)$. Combined with the β-decay constraint one finds an amusing consequence. The hydrogen atom is neutral; the charges of neutron and neutrino are opposite but not necessarily zero. So hydrogen is neutral, but heavy atoms are not if $Q_n \neq 0$.

Do grand unified models require equality of electron and proton charges? Conversely, would a difference in their charges, albeit small, rule out the grand unification programme? Okun *et al.* show that, in principle, grand unification is possible in such an eventuality. However the price is that there must exist a number of Higgs particles (these are predicted to be responsible for generating masses of W, Z particles and the fundamental matter field) so vast that their presence in virtual states would destroy standard low-energy electrodynamics. It is possible to avoid this catastrophe, but even so the models do not look very attractive.

Within the grand unified philosophy, then, it seems we must have $Q_e + Q_p \equiv 0$. The present limits, of the order of 10^{-20}, are tantalizing and it is by no means ruled out that an improvement could bring unexpected results. Indeed, experiments searching for evidence of fractional charge on niobium balls are using balls containing of the order of 10^{20} electrons and protons. If there is an imbalance in $Q_e + Q_p \sim 10^{-20}$ it would lead to a net charge on the ball that could mimic a fractional charge. This would not show up in experiments with oil or smaller metal drops, where as few as 10^{12} protons and electrons are sometimes present. It is possible that null results in these experiments could place strong limits on the electron–proton charge equality. □

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Cardiac physiology

A finger on the pulse

from A.F. Brading

In the determination of the mechanisms used by the heart to control its intracellular ions and regulate the rate and strength of its beat, the emphasis is shifting from descriptive accounts of factors affecting intracellular calcium to a more mechanistic approach in which the behaviour and control of the various processes involved is under scrutiny. So much was clear from a recent symposium* on the regulation of contraction in cardiac muscle.

The resting level of intracellular calcium is controlled by a combination of intracellular buffering and transmembrane pumps and leaks. Experiments on intact muscle and on isolated sarcolemmal vesicles reported at the meeting brought out the possibility that comparatively simple systems may underly the considerable complexities seen in intact preparations. From measurements with ion-sensitive microelectrodes of the intracellular activities of sodium and calcium and of membrane potential in sheep Purkinje fibres and ventricular trabeculea, in a variety of conditions, the stoichiometry required of a Na–Ca exchange for it to determine the intracellular free calcium has been calculated (H. Fozzard, University of Chicago). Fozzard finds that intracellular calcium adjusts so that the relationship between the sodium and calcium electrochemical gradients is unchanged, and fitted by a coupling ratio of 2.5 with a 'reversal potential' near the resting potential. J.P. Reeves (Roche, Nutley) has characterized an equivalent system in isolated vesicles of cardiac plasmalemma. It consists of a symmetrical exchange of one Ca^{2+} for three Na^+ in a direction determined by the calcium and sodium gradients and the membrane potential. He maintains that in normal resting conditions in the intact heart, the exchange will not be at equilibrium, and will mediate net calcium efflux even during membrane depolarizations to about 0 mV. These results suggest that other processes are more important than Na–Ca exchange in setting the level of intracellular free calcium, although the electrogenic exchange may contribute to the membrane potential under appropriate circumstances.

The rise in free calcium during the contractile cycle involves its entry through the membrane and release from the sarcoplasmic reticulum (SR). Calcium channels and transmembrane calcium currents can now be elegantly monitored with the patch-clamp technique, and R.W. Tsien (Yale University) illustrated the behaviour of voltage-sensitive calcium channels in normal conditions and in the presence of calcium antagonists and agonists. He suggested that the calcium channel operates in one of three modes, within each of which the channel may exist in one open and two closed configurations, with rate constants dependent on the membrane potential and the mode. Calcium antagonists bind preferentially to a mode in which channel opening is a rare event whilst the calcium agonists stabilize a mode in which the channel open-time is prolonged and the closed-time shortened. The mode existing most frequently in depolarized conditions is characterized by relatively short but fairly frequent channel openings. The interesting possibility was raised that there might be endogenous ligands which could modulate channel behaviour.

Calcium release from the SR is acknowleged to be one of the most important factors responsible for elevating free calcium levels. A. Fabiato (Virginia Medical College) has investigated its importance in mechanically skinned single cells with an exceedingly sophisticated set-up in which changes in the solution around the cell are made in a microprocessor-controlled and precisely timed sequence. The solutions contain aequorin, and its light signal and changes in tension are used to monitor calcium release. It seems that calcium entry during the spike triggers calcium-induced calcium release from the store, and depolarization of the SR membrane plays little part in the release process. Fabiato believes that the calcium is released via a calcium channel which is both activated and inactivated by free calcium on the outer surface of the SR. This means that release is not merely triggered by the level of free calcium but depends also on the rate of change of calcium activity. Inactivation starts at a lower concentration of free calcium than does activation, and with a much lower rate constant. Rapid application of supramaximal calcium levels will inactivate the process before release is triggered, but in the continuous presence of supraoptimal calcium concentrations another process of oscillatory calcium release is triggered (also described by D. Eisner, University College, London, in intact tissues with elevated intracellular calcium) which may trigger arrythmias in intact heart.

Once intracellular calcium is raised, its ability to activate the contractile machinery can also be modulated. For example, by correlating tension and intracellular calcium activity (measured using aequorin), J. Blinks (Mayo Clinic) showed that, following activation of catecholamine β-receptors, calcium becomes less effective at producing contraction,

*A Physiological Society Symposium on 'Myocardial intracellular regulation: consequences for the heart', University College, London, 28–29 March 1984.

whereas following α-receptor activation, the contractile apparatus becomes more sensitive to calcium. Activation of either receptor results in positive inotropy, but that produced by β-receptor activation is due to cyclic-AMP-dependent increases in calcium entry and calcium release from the SR.

The final meeting session covered clinical aspects. The underlying effects of hypoxia on mammalian hearts and the mechanisms of action of cardiac glycosides and the anti-arrhythmic agent lidocaine were discussed with reference to the involvement of the mechanisms discussed in earlier sessions. The possibility of developing more useful positive inotropic drugs was discussed by P. Poole-Wilson (Cardiothoracic Institute, London), but the meeting appeared to be somewhat pessimistic about this being achieved in the near future. □

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DNA replication

A twofold amplifed view

from David T. Denhardt

How does the mammalian cell accomplish orderly replication of its genomic DNA? Among several hypotheses, perhaps the most popular is the analogy with viral systems — that is, specific sequences in the DNA are activated in a regulated fashion by 'origin-activating' proteins, which cause replication to begin at what would then be considered replicon origins. Recently, a window onto this process has been opened as the result of studies on the replication of amplified, or amplifiable, replicons. In a well designed series of experiments, Mariani and Schimke have demonstrated the amplification of the gene for the enzyme dihydrofolate reductase (*DHFR*) in Chinese hamster ovary (CHO) cells in the course of a single cell cycle (*J. biol. Chem.* **259**, 1901; 1984).

During the normal cell cycle, the *DHFR* gene is replicated once in early S phase. When Mariani and Schimke blocked the entrance of a synchronized population of cells into S phase with hydroxyurea — an inhibitor of ribonucleotide reductase and so of DNA synthesis — replication of the *DHFR* gene was severely inhibited. Upon release from the block, the gene was rapidly replicated. More interestingly, and contrary to expectation, when replication was inhibited by hydroxyurea shortly after the cells had entered S phase, at a time when the *DHFR* gene had been replicated, within 12 hours the gene was replicated again.

The evidence is convincing that, within a single cell cycle, twofold amplification of the *DHFR* gene occurs in a substantial portion of the cell population. What this result suggests is that when DNA replication is inhibited by hydroxyurea partway through S phase, the cells revert back to their pre-S state. Hence, after removal of the block, the cells initiate replication of their DNA all over again. In effect, at least when the inhibition occurs in early S phase, the cell cycle clock is turned back to the boundary between the G_1 and S phases.

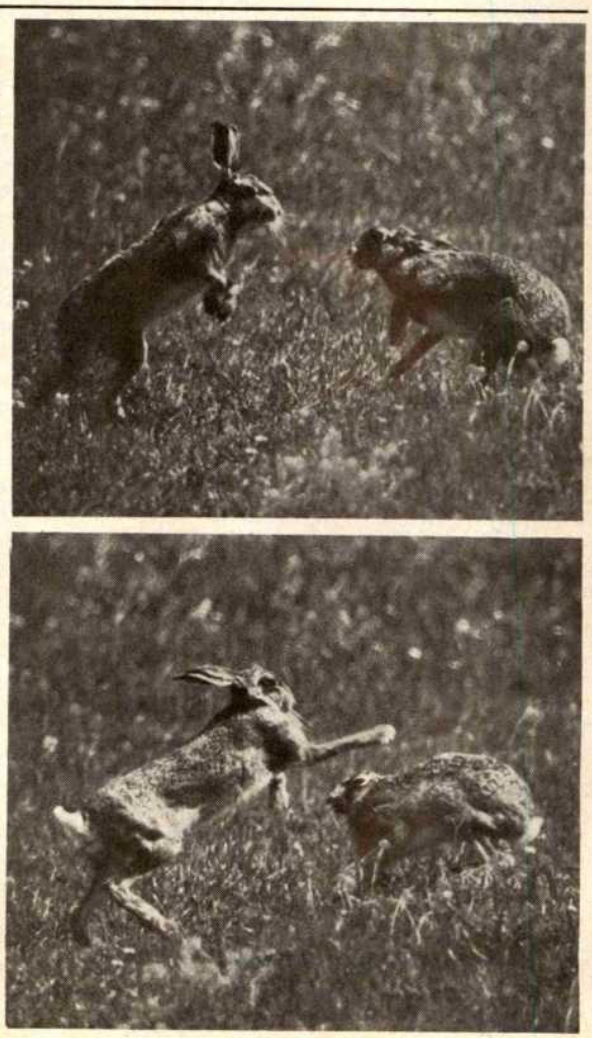
If an explanation for these events is to be sought in terms of 'origin-activating' proteins, one might infer that their stability is enhanced when DNA replication is inhibited to an extent sufficient to permit them to initiate a second round of replication within the same cell cycle. Programmed stability of an 'orgin-activating' protein would readily account for the amplification of the chorion genes of *Drosophila* follicle cells seen in the elegant electron micrographs obtained by Osheim and Miller (*Cell* **33**, 543; 1983).

Reinitiation at previously used origins during the same S phase has also been detected by, for example, Lavi, who demonstrated that when simian virus 40-transformed CHO cells are exposed to DNA-damaging agents (alkylating agents, aflatoxin) both the integrated viral genome and certain unlinked genes (*DHFR*, the *HLA* locus, c-Ha-*ras*) are amplified (*UCLA Symp. molec. cell. Biol.* **11**, 659; 1983). Moreover Barsoum and Varshavsky have shown that mouse 3T6 cells, which amplify their *DHFR* genes in response to the presence of the DHFR inhibitor, methotrexate, will do so with greater facility in the presence of tumour promoters and mitogenic hormones (*Proc. natn. Acad. Sci. U.S.A.* **80**, 530; 1983).

Support for the idea that there are specific origins of replication in mammalian DNA was reported last year by Hamlin and co-workers (*Nature* **302**, 439; 1983). They studied the replication in synchronized cells of the *DHFR* region in a CHO line carrying a 1,000-fold amplified *DHFR* gene (plus adjoining DNA) and found that replication of each of the 1,000 copies of the amplified DNA started in the same restricted region of the DNA and proceeded away from that origin in a bidirectional manner. Although the origins of replication have not yet been localized to a defined nucleotide sequence, this will surely soon be accomplished. □

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CAMELIA VS CADET



On her travels in Wonderland, Alice was given the choice of heading in the direction of a Hatter or a March Hare. She chose the latter because "The March Hare will be much the more interesting, and perhaps, as this is May, it won't be raving mad — at least not so much as it was in March". Lewis Carrol, and Martin Gardner after him in *The Annotated Alice*, were wrong to ascribe such a specific seasonal insanity to the Brown Hare, according to 1,500 hours of observations by Anthony Holley and Paul Greenwood summarized on page 549 of this issue. The mad behaviour of hares is continuous throughout their long breeding season which lasts from January to August, and is no more frequent in March than in any other month of that season. Equally wrong, they conclude, is the traditional belief that the pairs of hares seen madly boxing are both males, and that the boxing represents intrasexual competition. On the contrary, boxing is between a female and an unwanted suitor: here Camelia is seen to be getting the better of Cadet.

Biopolymers

Silken spider chains

from Paul Calvert

SILK is best known as the fibre spun by the larvae of many moths to form a protective cocoon for pupation; most commercial silk is from the domesticated larvae of the moth *Bombyx mori* with some 'wild' silk from the Tussah silkmoths *Antheraea*. A great variety of other insects also make silks, fibres of which find uses as supports, shelters and attachments, but the real silk specialists are spiders. Despite the efforts, in the eighteenth century, of M.Bon of Montpellier, who made himself stockings and gloves from spider silk, it has caught on neither commercially nor scientifically. Research papers on spider silk, such as that by Gosline *et al.* on page 551 of this issue, are therefore rare treasures.

Most silks are proteins, containing a predominance of glycine, alanine and serine. For instance, *B. mori* silk is close to a simple repeating (Gly-Ala-Gly-Ala-Gly-Ser) structure. By comparison, spider dragline silk stands out in having 10 per cent each of proline and glutamic acid. In properties and structure, the silks are first cousins to the nylon fibres. Commercial nylons, however, do not have such a high density of amide units as the silks because it makes for strong hydrogen bonding, which results in a melting point too high for processing and a fibre that is too water-sensitive. A shirt that becomes elastic when wet could be a little discomforting; but for animal silk, hydrophilicity is essential as it permits the fibre to be spun from aqueous solution.

The paper of Gosline *et al.* builds on the work of Work and colleagues who showed there to be a 50 per cent contraction, along with a twofold volume increase, when spider dragline fibres are allowed to swell in water (*Textile Res. J.* **47**, 650; 1977 and **52**, 349; 1982). (The Work papers are worth reading at least for the description of how to milk a spider of its dragline.) This water sensitivity is particularly strange in that the dragline must support the falling spider and yet the twinned lines of the garden spider, *Araneus diadematus*, which can weigh 0.65 g, break at 1 g (see F. Lucas *Discovery* **25**, 20; 1964; Lucas & Rudall *Compreh. Biochem.* **26B**, 475; 1968). Such a close strength specification seems to leave little room for rapid deceleration and none for unpredictable properties.

Gosline *et al.* show that the uptake of water converts the amorphous regions of the fibre to a rubbery state. Silks, like synthetic fibres, are composites of small crystalline regions and rigid glassy amorphous regions. For spider silks, no information on the proportions of the two phases seems to have been published, but most natural and textile fibres are at least 50 per cent crystalline. On swelling in water the glassy regions become plasticized so that the chains are mobile, and possibly some of the crystals melt. The result is that the structure relaxes, the chains in the amorphous regions coil up and the modulus decreases by a factor of 1,000. If the fibre is stretched and dried, the original properties return. This process is similar to heat shrinkage of synthetic fibres; for instance, drawn polyester fibres, when heated to 240°C, shrink by 75 per cent and the modulus drops 50-fold (M.P.W. Wilson *Polymer* **15**, 277; 1974).

The size of the modulus drop in spider silk is surprising. The final modulus of 10^7 Pascals is more like that of true rubber than that of a partly crystalline polymer — the modulus of a 50 per cent crystalline polyethylene is 50 times higher. This suggests that the water-resistant crystallinity of spider silk is very low and that most of the dry fibre is oriented glassy material. A reasonable guess would be that the high proline and glutamic acid contents are responsible for preventing much crystallinity developing. This appears strange when the spider could have produced a strong water-resistant fibre of the silkmoth or nylon type. What the spider gains is a fibre that is about twice as strong as *Bombyx* silk and has a very much larger extension (30 per cent) before it breaks. This extension will gradually slow the falling spider rather than there being a thread-breaking jerk. The penalty, apparently, is a rather disconcerting change in behavior in the rain.

Synthetic fibre makers should be impressed by the range of properties attainable within the silk group and spurred in their efforts to control polymerizations to the point where silk-like structures can be duplicated. As a general rule it is very hard to increase the strength of a material without also decreasing the extension to break; the way that both are improved at once in spider silk, compared with silkmoth silk, is impressive. □

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Cell physiology

Cellular site of calcium regulation

from Andrew P. Somlyo

THE concentration of free (ionized) calcium (Ca^{2+}) regulates not only muscle contraction, but also a variety of other cellular processes including glycogen metabolism, secretion, membrane transport and permeability. In muscle, an intracellular organelle system, the sarcoplasmic reticulum (SR), can store and release calcium, and so regulate cytoplasmic Ca^{2+} in the absence of a change in total cell calcium. But what plays the part of the SR in non-muscle cells? The suggestion that it is the mitochondria seems to have stemmed, in part, from the great attention given to mitochondrial Ca^{2+} transport[1,2]. When exposed to high (unphysiological) concentrations of Ca^{2+}, mitochondria can accumulate very large amounts of calcium phosphate salts, nearly enough to be turned to stone. As known since the time of Lot's wife, this is an eye-catching device, but not conducive to much further activity. Here I shall summarize some of the compelling arguments why opinion is shifting strongly away from mitochondria towards the endoplasmic reticulum (ER) as the regulator of cell calcium.

Subcellular fractions containing ER and showing Ca^{2+}-accumulating activity have now been isolated from a wide variety of tissues[3-10]. In some instances, contamination by ER vesicles may account for active calcium uptake attributed to some other organelle, such as synaptic vesicles[11] and, perhaps, vertebrate retinal discs. The ER calcium pump resembles the SR system in using ATP (although other high-energy phosphates can substitute) as an energy source, in being enhanced by calcium-precipitating anions such as oxalate and phosphate and in being inhibited by SH-reactive organic mercurials[10]. Moreover, a phosphorylated 118,000-molecular weight protein, thought to be analogous to the phosphorylated intermediate of the SR Ca-ATPase, has been identified in ER from rat liver[12]. The affinity of several isolated ER preparations for Ca^{2+} is sufficiently high ($K_m \sim 0.2$–$0.6\mu M$) to reduce cytoplasmic Ca^{2+} to the low levels typical of normal resting cells. It has yet to be determined whether there are specialized regions of the ER with a high capacity for storing calcium, like the terminal cisternae of striated muscle SR. The identity of the subfractions of ER largely responsible for calcium-accumulating activity is not known, although it is probably not inherent to the rough endoplasmic reticulum[6].

The introduction of new techniques has greatly advanced the identification of the ER as the source and sink of intracellular calcium in non-muscle cells. For example, whereas squid axons are sufficiently large to permit the manipulation of their intracellular Ca^{2+} by internal perfusion[13,14], for smaller cells, membrane permeabilization by agents, such as saponin, has been used to manipulate intracellular Ca^{2+}. This has allowed investigators to identify the ER as

a calcium storage site, by showing that Ca^{2+} accumulation occurs even at low Ca^{2+} concentrations at which mitochondria cannot accumulate calcium or when mitochondrial calcium uptake is inhibited[10,15]. Thus ER in liver cells, synaptosomes and macrophages has been shown to accumulate Ca^{2+} at the low concentrations required of a physiological regulator. A more direct approach has been to visualize, through electron microscopy, sometimes combined with electron-probe X-ray microanalysis, the deposits of calcium (or strontium, which acts as a more electron-opaque substitute for calcium) oxalate in the ER of synaptosomes[5], squid axons[13], photoreceptor cells[16] and endothelium[17].

For the ER to regulate cell Ca^{2+}, it must have not only a mechanism of calcium uptake, but also one of calcium release. On stimulation of cells by hormones or transmitters, the signal for Ca^{2+} release may be mediated by a change in the properties of the surface membrane, most commonly depolarization, and/or by a diffusible intracellular second messenger. The feasibility of the first mechanism is suggested by the close (about 10–20 nm) proximity of ER subsurface cisterns to the plasma membrane. These structures, which resemble the 'surface couplings' of cardiac, smooth and some skeletal muscles, have been observed in some non-muscle cells[18-20] and may be present in all eukaryotic cells. There is also evidence that electrical stimulation of the surface membrane of fibroblasts, known to contain subsurface cisternae, can release calcium from an intracellular source, probably the subplasmalemmal ER[20]. The second possibility, that an endogenous intracellular messenger releases calcium from the ER, has recently received strong support. Thus in pancreatic cells[21], in isolated hepatocytes[22] and in insulinoma cells[23], a phosphatidylinositol metabolite, inositol trisphosphate, has been shown to release intracellular Ca^{2+} from a non-mitochondrial site, presumably the ER. It was further observed that caffeine releases intracellular Ca^{2+} from a non-mitochondrial source in rat nerve cells[24], reminiscent of the caffeine-induced Ca^{2+} release from the SR of skeletal muscle[25]. Caffeine-induced Ca^{2+} release from the ER has also been observed in macrophages[8], but not in liver[15].

The first inkling that mitochondria might not have a significant role in the regulation of cytoplasmic Ca^{2+} followed the observation that, in the presence of physiological Mg^{2+} concentrations, the affinity of isolated mitochondria for Ca^{2+} is too low[26]. In the normal cell, Ca^{2+} fluctuates, at most, from 5×10^{-8} to 5×10^{-6} M between rest and maximal activation, while the Ca^{2+} concentration required for a half-maximal rate of calcium transport by mitochondria is 10^{-5} M or higher[17,26]. Furthermore, in intact cells, the calcium content of mitochondria is relatively low compared with that of the SR and even after sustained increases of Ca^{2+} within the physiological range in maximally contracted muscle, mitochondrial calcium content is not increased[17]. On the other hand there is no question that in the presence of inorganic phosphate (P_i), mitochondria can accumulate massive amounts of calcium and, perhaps, become initial sites of pathological calcification when cell Ca^{2+} (and P_i) rises to abnormally high levels due to cell damage. In injured cells, mitochondria may serve as the final low-affinity/high-capacity buffer for Ca^{2+}, and moderate loading of mitochondria by Ca^{2+} may be an alternative to cell death. It remains to be shown that such cells survive and that mitochondria can discharge their excess Ca^{2+} to the extracellular space via the various surface-membrane-mediated calcium transport mechanisms that must ultimately maintain the steady-state calcium content of cells. One candidate for a naturally occurring 'transmitter' that can release mitochondrial calcium has been identified: sodium can discharge calcium from mitochondria isolated from heart, brain and skeletal muscle, although not from liver, kidney and smooth muscle mitochondria[2].

What, then, is the physiological role, if any, of the complicated mitochondrial calcium transport system so passionately pursued by biochemists for nearly 20 years? One possibility is that, although mitochondria do not have the capacity to regulate cytoplasmic Ca^{2+}, they themselves can respond to changes in cytoplasmic Ca^{2+} concentrations by producing very small changes in the Ca^{2+} of the mitochondrial matrix space[27]. A number of the mitochondrial enzymes (dehydrogenases) are sensitive to Ca^{2+} in the range 0.1–10 μM. Therefore, small changes in mitochondrial Ca^{2+}, achievable by even a relatively inefficient (low-affinity) calcium transport system, could have important effects on oxidative metabolism.

While far from complete, the case (even in plants) for the ER as the major intracellular regulator of Ca^{2+} has clearly become much stronger during the last few years. The lumen of the ER communicates with the perinuclear space where, in some instances, high concentrations of calcium (or strontium) have been demonstrated. Perhaps an ER system, capable of accumulating calcium against a gradient in the nuclear membrane, was acquired with the nucleus when cells became eukaryotes. Unfortunately for biologists, living systems often evolve to use different structures and mechanisms to solve the same problems (photoreceptor systems and contractile regulation are but two examples), and grand unifying schemes with fundamental principles applicable to 'all cells' are rare. In the regulation of cell Ca^{2+} by the ER in all nucleated cells, we may have a rare example. □

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100 years ago

ARRANGEMENTS have been made by the Council of the Scottish Meteorological Society for the completion this season of the Observatory of Ben Nevis. The first portion of the Observatory was, it may be remembered, opened in October last, and since the observers went into residence continuous hourly observations have been made of the conditions of the atmosphere at the top of the Ben, with special reference to temperature, pressure, humidity, and motion. From the discussion of these, and what were daily made by Mr Clement L. Wragge in the summers of 1881 and 1882, by the Secretary, Mr Buchan, the Council have been fully confirmed in the high expectations they had formed concerning the value of a high-level station, both in its bearing upon general meteorological problems, and also with reference to possible forecasts for the British Islands. The additions to be made to the Observatory will just double its size, and enable the three observers — who during the winter have been considerably cramped in their one apartment — to work under more comfortable conditions. On the south of the present doorway there is to be erected a shelter for tourists. The estimated cost of the completion of the Observatory will be 800l., which is, however, irrespective of a heavy item of charge for conveying on horseback the materials to the top of the hill. It is understood that the cost of equipment and maintenance of the Observatory heretofore has been heavier than was anticipated. The directors intend shortly to make a fresh appeal for funds to the public, which will no doubt be as liberally responded to as was their last.

From *Nature* **30**, 179, 19 June 1884.

Mutations and oncogenes — cause or effect

SIR — In a field as complex as carcinogenesis, it is difficult to distinguish between cause, correlation and consequence. No thoughtful biologist needs to be reminded of the many cancer theories propounded in this century. The current version is represented by the flood of papers on oncogenes and karyotypic abnormalities as the underlying cause of cancer, with amplification in the popular press.

The problem in unqualified acceptance of the claims can be illustrated by an examination of some details of specific and nonspecific chromosome changes in cancer. The most thoroughly investigated of the specific or nonrandom changes is the Philadelphia chromosome (Ph^1) associated with over 90% of human cases of chronic myelogenous leukaemia (CML)[1-3]. However, there are patients with typical CML whose leukaemic marrow cells are Ph^1 negative at first, but become Ph^1 positive later[4]. In one case, Ph^1 negative and Ph^1 positive cells were observed in the same clone of leukaemic cells[5]. The evidence suggests that blood cells remain karyotypically normal but unstable for some time after the initial malignant transformation but provide a milieu favourable for the establishment of the Ph^1 anomaly[5].

Similar evolution of nonrandom chromosome alteration occurs in sarcomas induced in rats by infection with the Schmidt–Ruppin strain of Rous sarcoma virus. More than 80% of the tumours have a normal diploid stemline[6]. In some cases, all the cells are normal diploid while in others there are a few near-diploid sidelines or pseudodiploids. In older tumours, however, heteroploidy is common and usually involves the specific, sequential addition of three particular chromosomes. Without a careful impression that the chromosonal aberrations are a prerequisite for tumour formation since the predominance of normal diploid cells in early tumours is sometimes not noted when the nonrandom changes are discussed[7].

Many of the reports of nonrandom chromosome changes or activated oncogenes in tumours are based on studies of cultured lines of the tumour cells. The caution that must be used in interpreting them is illustrated by a study of eight human gliomas[8], each of which had a unique and broad distribution of karyotypes ranging from hypodiploid to as much as hypertetraploid when first placed in culture. Reference sets of frequent karyotypes were established for each of the tumours, only 7 to 25% of the clonal karyotypes were represented in the reference sets. Thus in no case were the cells which were maintained in culture representative of the original tumour, and any conclusions drawn from their analysis could not be applied to the bulk of the tumour cells. All of the examples given demonstrate that progression within the tumour and selection in culture obscure the relationship between cause and effect.

Chromosome studies have an advantage over molecular analysis of oncogene analysis because karyotypes can be obtained of individual cells, and a population distribution determined, whereas the biological and chemical methods used to identify oncogenes represent an average of the whole of the population. Furthermore, karyotype analysis is technically simple and has been pursued even in clinical laboratories for many years, so it has provided a depth of information, particularly in human cancer, not yet available in oncogene studies.

There are, however, hints that the questions raised about the role of chromosome changes in tumour development have their parallels in studies of oncogenes. Most of the positive reports of oncogene activation derive from transfection studies with DNA from cell lines[9]. (Chemical identification of activated oncogenes in some 50 reported cases of human cancer has been negative[9]). The most prominent cellular oncogenes are related to acute viral transforming genes which are known to give a selective advantage for cell growth in culture. The possibility must therefore be considered that cell structure selects for a minority mutant cell in the original tumour population. The activated oncogene would then be no more representative of the tumour cells than was the clonal karyotype in the glioma representative of the tumour karyotype.

It has recently been shown that a human osteosarcoma cell line which was negative on transfection to NIH3T3 cells could become positive after treatment with *N*-methyl N'-nitro-*N*-nitrosoguanidine (MNNG), a mutagen and carcinogen[10]. The chemically-transformed cells were more transformed in appearance than the original cancer cells and produced more tumours in nude mice. Obviously the "oncogene activation" was an epiphenomenon unrelated to the original tumour. Although this activation may have played a role in the secondary transformation of the tumour cells in culture by MNNG, even this is uncertain, since a similar enhancement of the transformed phenotype in the same cancer cells by another carcinogen was not accompanied by detectable onocogene activation. Many more inconsistencies in the ever burgeoning oncogene literature could be cited to raise suspicion about their purported role in carcinogenesis (see ref. 9). At the most, their role in tumorigenesis appears to be auxiliary[11], at the least inconsequential.

As confirmed recently in *Nature*, the initial effects of carcinogenic treatment affect the entire population of exposed cells[12] and therefore cannot be genetic. The rarer, random occurences which characterize the secondary stages of transformation[12] can be explained as well by differentiative[13] as by mutational events. Indeed, the great mass of (largely dormant) evidence which indicates that cancer is the far end of a spectrum of development of cell and tissues[14,15] seems to be only selectively disinterred to rationalize gaps in mutational theories of cancer. The lack of an adequate mechanistic explanation for developmental processes and their derangement is no justification for ignoring them.

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Oligodendroglia in multiple sclerosis

SIR — The report by Ludwin[1] that the oligodendrocyte proliferates around an injury to mouse brain is of great relevance to previous studies on the neuroglia in mutliple sclerosis (MS). Some twenty years ago, we demonstrated proliferation (or at least increased numbers) of oligodendroglia at the edge of active MS plaques[2,3]. This observation has recently been confirmed by electron microscopy[4].

Although oligodendroglia are essentially absent from the centre of MS plaques[2,3,5], their proliferation at the edge of the lesion suggests either a response to injury or their involvement in repair of the damaged myelin. Ludwin's dynamic isotope study clarifies the controversial issue of the oligodendrocyte's potential to undergo hyperplasia and possible role in repair.

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Archaeomagnetic dating of Santorini volcanic eruptions and fired destruction levels of late Minoan civilization

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Archaeomagnetic dating on the Minoan ash horizons of the Santorini volcano and on fired destruction levels at late Minoan sites on Crete demonstrates that the basal (Plinian) air-fall ash of the first 'Minoan' pumice is contemporaneous with the destruction levels on central Crete, while the higher 'Minoan' ashes are contemporaneous with the destruction levels in extreme eastern Crete. These destruction levels were almost certainly caused by seismic activity, rather than the ash fall. The determination of a time gap between these events lead to a reappraisal of the archaeological evidence and is important volcanologically.

THE excavation of a highly advanced Bronze Age culture in Crete and surrounding islands at the turn of this century, led to speculation[1] that this Minoan civilization was the basis of some Homeric legends and also of Atlantis. Further excavations apparently indicated that the widespread devastation and extensive burning of towns, palaces and isolated villas were synchronous[2], and that this appeared to be simultaneous with the eruption of the Santorini (Thera) volcano, 120 km north of Crete (Fig. 1). Marinatos[3] proposed that there was a direct relationship between the two events and others, such as Luce[4], have shown how such an association is consistent with features of the Atlantis legend. It has also been shown[5] how the volcanic activity could account for the Egyptian plagues and events that occurred as Moses was leading the Israelites out of Exile.

The distribution of ash in the Eastern Mediterranean and seasonal wind patterns suggest a summer eruption[5,6] and Biblical references[7] also indicate late spring or early summer. The ashfall could thus have directly destroyed growing crops. The ash clouds may have contained fluorine[8]. It has also been suggested[9,10] that tsunamis destroyed the Minoan fleet, leaving the area open to invasion. However, objections have been raised to such suggestions particularly as they depend on the eruption of Santorini being synchronous with the destruction levels. Unfortunately no dating method is sufficiently accurate to determine the date of either event. Dating by ^{14}C has given a wide variety of ages[11–15]—the most widely accepted are between 1700 and 1200 BC, mainly because such a range is broadly consistent with the archaeological evidence[16]. This is based on wall paintings in Egypt, contemporaneous with Thotmose III, which depict envoys from Crete attributable to late Minoan IB (LMIB) times. LMIB vases in Egyptian tombs of this age indicate that Cretan LMIB time corresponds with Thotmose's reigh, 1503–1447 BC[17].

Acidity layers in the Greenland ice sheet, attributed to pollution of the atmosphere by the Santorini eruption, have a calculated age of 1390 BC[18], while frost damage in tree rings of the bristlecone pine in southern California have been dated as 1626 BC[19]. If *Exodus*[20] does record the Santorini volcanic events, this was 480 yr before Solomon's fourth year: 1446 BC, although other dates have been suggested, such as 1250 BC (J. V. Luce, personal communication). There is thus no consensus of any one date for the volcanic eruption.

Archaeomagnetism[21,22] has conventionally been used as an 'absolute' dating method, based on the fact that fired materials are capable of retaining a magnetic remanence so that the direction and intensity of the geomagnetic field can be determined for the time that the materials were cooling. For periods when the directional and intensity changes are known, such fired objects can be dated by comparing their properties with that of the previous geomagnetic field. Advances have been made in establishing such geomagnetic secular variation records in many parts of the world, with results from Bulgaria[23], in particular, extending the knowledge of the geomagnetic field in southeastern Europe over the probable age of the Minoan eruptions and destructions. However, such records are still sparse, their ages largely based on ^{14}C determinations which means that no absolute time scale for secular variation during the Minoan period has been established. However, relative dates can be determined. Materials magnetized at the same time in the same region will normally have identical directions and the intensity of the geomagnetic field would also be the same. It was on this basis that the archaeomagnetic study of the Santorini volcanics was initiated[24] and extended to late Minoan sites on Crete[25] (see Table 1).

Collection and analysis

To establish synchroneity, sufficient samples had to be collected, to allow averaging out of random errors, and had to be measured with great care. The ashes were particularly difficult to sample as most are either unconsolidated or only loosely consolidated. Collection was by the disk method[22], in which 2.5-cm plastic disks are glued to the sample, oriented and removed, with the sample attached, for measurement in a version of the Digico magnetometer[26]. The flat, upper surface of the disk was oriented with an inclinometer and, wherever possible, a Sun compass. Unfortunately some archaeological sites were protected by a roof so that magnetic compass orientation was necessary on some occasions. The individual orientations are considered to be accurate to about 1°, comparable with the instrumental measurement error, so that each sample error is thought to be of the order of 1–2°. The identification of individual components within any one sample is less precise but such errors should be random and are thus reduced by averaging. The ancient intensity of the geomagnetic field in which the geological and archaeological materials originally cooled was determined by a modified Kono and Ueno[27] method.

The magnetic minerals were identified using isothermal magnetic properties, X-ray diffraction, Curie temperature analysis, microprobe, thermal and alternating magnetic field studies. It was found that almost pure magnetite was the dominant remanence carrier in both archaeological and geological

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Table 1 Details of Cretan late Minoan sites

Site	Area	Description and location of sampled areas	Usable samples
Malia	Palace (13) House ($\Delta\alpha$)	Area to the east of L'entree sud; samples burnt mud-brick from walls of low elevation. Room 7, purification chamber; inner part of NNE–SSW wall. Area 5—'block-wall' running parallel to wall of room 7 (hard 'vitrified' sandstone). Wall to east of rooms 10 and 11. Room 8, small partition mud-brick wall (magnetic compass).	20 24
Knossos	Stratigraphical museum extension site kiln 2	Upper kiln walls and kiln chamber end of channels: circular sampling distribution (magnetic compass).	26
Sklavokambos	Villa	Room 18 to west of small central court (possibly storeroom or kitchen). Interior of east wall dividing rooms 18 and 15. Room 10—burnt mud-brick and limestone samples.	16
Phaestos	Palace	3 areas: (1) area 101 beyond NE area of Palace (burnt mud-brick partitions). (2) Area to north of central court (open paved corridor[41]) vertical burnt plaster walls on east sides of corridor 41. (3) North wall of room 70 to NW of central event (possibly a lustral basin or guard room).	17
Hagia Triada	Palace	Areas near room 8 and 12—east facing wall at bottom of staircase (scala 60), burnt floor at base of staircase to west of room 8, west interior wall of room 8 at top of scale 60, north exterior wall of room 12.	17
Gournia	Town House Ac	Room 16—large floor level mud-brick wall—samples from top to bottom exterior and interior of wall were exposed.	30
Makrygialos	Villa	Samples of burnt mud-brick from low elevation walls (all orientations) and pillar bases. Also floor samples.	38
Palaikastro	Town (Block N)	Burnt sandstone and mud-brick from low elevation walls (block N). Burnt floor from compartment 9.	59
Kato Zakros	Palace	NE annexe of palace, room N; NE–SW wall, pantry LI; staircase of the shrine; inner N wall of pantry, stores L; inner E (NE–SW) wall, pantry store L; inner E (NE–SW) wall; pantry stores LI. Outer S (SE–NW) wall, pantry store L; N wall, room XI E of door; N wall, room XI, W of door; NE–SW wall, room XII (inner) to W of steps: NW–SE wall, room XI, E of door; NW–SE wall, room XV, W side; NW–SE wall room XV, SE corner; N edge of wall extending NE from room XXVI; NE–SW wall, room XXVI (W edge), NW–SE wall, room XXV (treasury); NE–SW wall, room XXV (above archive niches); doorway of N wall between rooms XXVII and XXVI. (All burnt mud-brick.)	49

samples, occurring within rapidly chilled minerals within a glassy matrix in the tephra. Subordinate titanomagnetite (X = 0.4; Curie temperature 420 °C) occurs as early phenocrysts in the tephra. The magnetic behaviour[28] indicated that the remanence was carried by either single domain or pseudo-single domains. The archaeological materials, mostly burnt mud bricks from large *in situ* structures, had been heated to temperatures above the Curie point of their magnetite. However, even unconsolidated charcoal ashes taken from floors (Hagia Triada palace and Palaikastro block N) were found to contain a stable remanence with identical directions to those of the associated burnt mud brick walls.

Results from Santorini

Any interpretation of the magnetic data from the volcanic deposits requires an understanding of how their magnetization was acquired. If they were emplaced at a temperature below that of their Curie temperature, then at least two primary components would be present—one acquired before and one after emplacement. Higher reliance can be placed on directions from a single-component system and palaeointensity determinations from a multicomponent system will be particularly difficult to evaluate. Samples of Minoan tephra were taken from locations on Santorini, ranging from close to the vent (Thera quarry) to those overlying the Minoan city of Akrotiri, 7 km from the former vent (Fig. 2). Analysis of the components, similar to that described by Hoblitt and Kellogg[29], yielded emplacement temperatures of 300 °C for both the Plinian ashfall and the base surge deposits at Akrotiri, and significantly higher values, ~500 °C for both layers, nearer to the vent. The tephra deposits overlying the base surge at Thera quarry were emplaced at lower temperatures, 300–400 °C, showing that these originated as ash flows and not mud flows[30]. The overlying ignimbrites, sampled at several sites on the eastern coast of Santorini, yielded higher temperatures, above 500 °C. (These emplacement temperature results will be discussed in detail elsewhere[31].)

The presence of two primary components of remanence in many of the ashes overlying the base surge and below the ignimbrites meant that careful analyses were required to separate the two components, and it was frequently the value of the component being removed during partial demagnetization that provided a more accurate definition of the lower temperature component than the component remaining. The ignimbrites and base surge deposits in the quarry were characterized by a single primary component and could be analysed more simply, although these were also subjected to both alternating magnetic field and thermal demagnetization, determination of linearity, planarity, and so on[32]. These analyses generally demonstrated extremely high stability using any of the standard measures, such as the stability index[33], although 25% of the volcanic samples had to be rejected where the different components could not be adequately separated. Most difficulty was encountered in the ashes immediately overlying the base surge deposits but excellent results were obtained from the Plinian ashfall, the base surge and the overlying ignimbrites. The geological and geochemical evidence indicates that the base surge, overlying choatic tephra and ignimbrites are essentially part of the same eruptive sequence[34] and therefore the less well-defined results from the chaotic ashes make little difference to the final conclusions.

After removal of viscous, short-term components by partial demagnetization, the directions of the component acquired at, and just below, the emplacement temperature were combined for the different volcanic units. This showed that the Plinian ashes were characterized by distinctly steeper inclinations than observed in the overlying ashes and ignimbrites, but all overlying tephra showed mutually consistent directions (Table 2, Fig. 2). The observed difference clearly indicates an age gap between the Plinian and base surge deposits, although this had not been

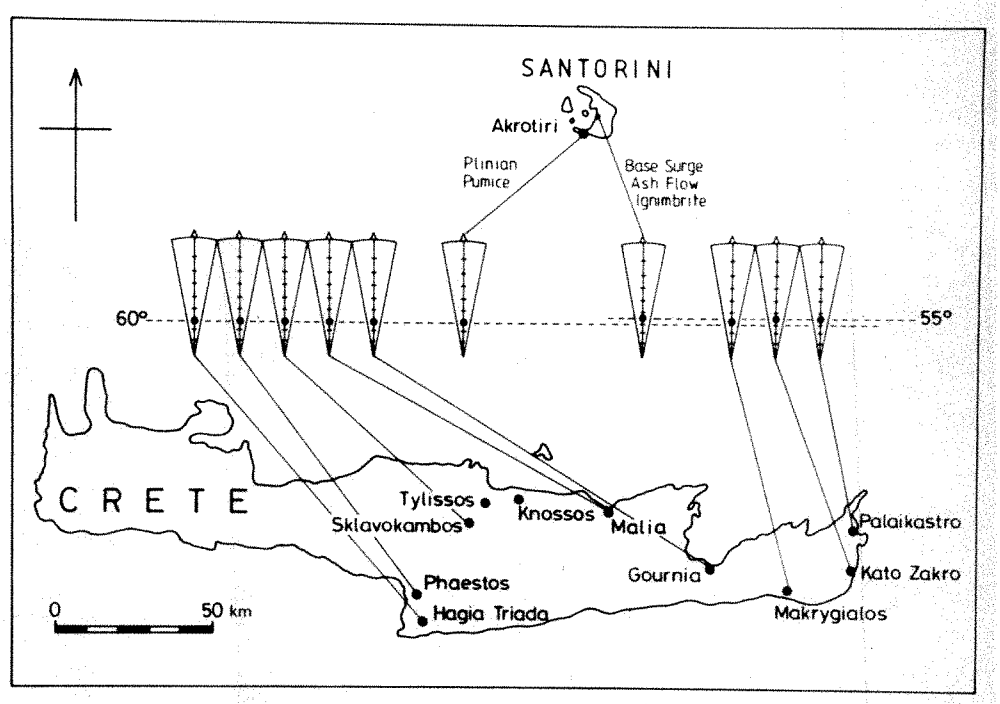


Fig. 1 Map of the sites and mean directions on Crete and Santorini. The mean directions are illustrated by means of a segment of an equal-area stereographic projection. In this projection the central line indicates north and the inclination varies from zero on the circumference to vertical at the centre. The mean direction is shown as a dot, the size of which is already greater than the 95% precision parameters associated with it. The declinations are more westerly for the projections on the left, but are not very different from those of the other projections. However, the inclinations are statistically steeper on the left than those on the right and this difference is supported by the available palaeointensity determinations (Table 2).

expected from previous geological and geochemical studies[34]. These showed little or no difference between the composition, refractive indices, and so on, of the Plinian ashes and the bottom of the base surge. Geochemical changes from within the base surge deposits into the overlying ashes were not considered significant as the base surge and overlying chaotic tephra were clearly part of the same volcanic sequence of events. Closer examination of these data indicates that the similarities between the Plinian ashes and the base surge are restricted to the very basal layers of the surge deposits and could be explained if some Plinian ashes were incorporated in the surge as it scoured across their surface. We suggest that geochemical differences may exist, consistent with the magnetic evidence for a significant time gap between the Plinian ashfall and the base surge events. (These new magnetic results differ from those originally obtained from the Plinian ash and base surge deposits[24], but previous sampling methods had restricted the collection to volcanic bombs which are now known not to be characteristic of the main tephra deposits.)

The only archaeological site which could be studied on Santorini was a small hearth structure at Akrotiri (Arvaniti 1 α 1). Unfortunately the roof over the site prevented the use of a Sun compass and the large pithoi nearby may have affected the magnetic orientations. This could account for the higher scatter ($\alpha = 3.6°$) than observed at most other archaeological sites examined in this study. The mean direction differed from those of all of the overlying ashes (Table 2), although this difference was not statistically established at a 95% probability level. However, the palaeointensity determinations on both the hearth and some pottery sherds also indicated an age difference.

Table 2 Directions and palaeointensities of geomagnetic fields recorded on Santorini and Crete

	Mean directions				Palaeointensity (10^{-4} T)		
Site	Decl. (deg)	Incl. (deg)	α_{95}	No.	Intensity	s.d.	No.
Palaikastro (block N)	355.1	55.1	1.8	59	0.530	0.030	9
Kato Zakro (palace)	355.8	54.6	1.9	49	0.607	0.024	6
Makrygialos (villa)	354.7	57.5	1.4	32	0.619	0.057	5
Paroxysmal tephra	0.5	54.8	2.0	92	—	—	—
Gournia (house Ac, 16)	356.1	59.5	1.0	24	0.634	0.008	6
Malia (palace area 13)	356.1	59.6	1.4	20	0.617	0.026	7
Malia (house $\Delta\alpha$)	356.6	59.9	3.2	22	—	—	—
Knossos (kiln 2 mus. ext)	355.2	60.8	1.6	25	0.615	0.067	3
Sklavokambos (villa)	354.3	59.1	3.9	16	0.692	0.027	5
Phaestos (palace)	356.5	61.1	1.7	17	0.673	0.066	4
Hagia Triada (palace)	353.8	60.1	2.3	17	0.681	0.020	6
Plinian ashfall	359.7	61.1	3.2	40	0.670	0.100	6
Akrotiri (hearth α1)	10.6	57.3	3.6	17	0.510	0.049	6
Akrotiri (pottery)	—	—	—	—	0.578	0.072	5

Cretan results

Ten Minoan excavations were visited on Crete (Fig. 1), and their final destruction levels were sampled. To detect any deviations caused by anisotropy or magnetic refraction, samples were taken from walls in different orientations and, in the case of kilns, systematically around the wall linings (see Table 1). No evidence was found for either magnetic refraction or anisotropy. The age of each site was ascertained by consultation with local archaeologists and from literature. In all the cases, the fired levels were considered to represent the 'final' Minoan destruction levels. (At Knossos, habitation continued long after the cessation of the Minoan civilization.) These levels have largely been dated on the basis of associated pottery using, for example, Popham's classification[35] and are generally regarded as LMIB, corresponding to the presence of a 'marine' style of pottery, and therefore younger than late Minoan I A (LMIA) with a characteristic 'floral' style, although this style persisted into LMIB times. There is controversy about the stylistic classification of some isolated pots and the absence of LMIB pottery does not mean that none will be found or that the extant pottery style at one location was not contemporaneous with LMIB styles as defined at Knossos. At Malia palace, for example, few pots of confirmed LMIB have been found (O. Pelon, personal communication) while LMIB pottery is abundant at Palaikastro.

The presence of single primary magnetic components of very high stability, carried by single or pseudo-single domain magnetite, allowed very precise definition of the directions at each locality. The directions at the sites in eastern Crete (Palaikastro and Kato Zakros) were found to be identical, with the mean

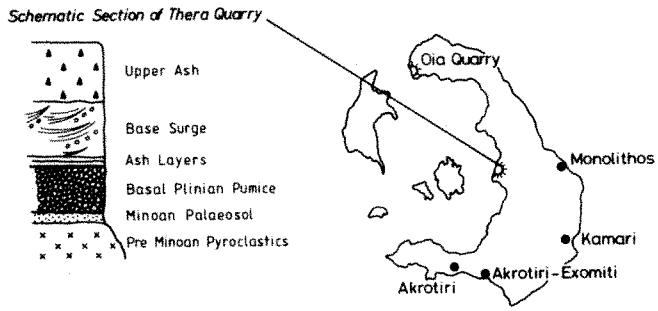


Fig. 2 Sampling sites in Santorini. Most of the tephra sampled were from the sequence exposed in the Thera quarry.

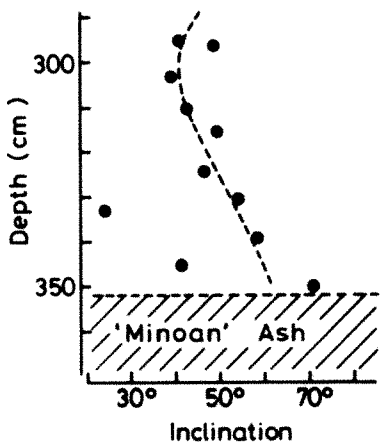


Fig. 3 Changes in the geomagnetic field as recorded in eastern Mediterranean sediments. New magnetic studies[25] of the VEMA V10-50[5] core of the eastern Mediterranean sediments show a decrease in inclination with time in the carbonates overlying the Minoan tephra, consistent with the archaeomagnetic studies on Santorini and Crete.

inclination at the other eastern Crete site, Makrygialos, being only slightly steeper. The remanent directions at sites from central Crete (Malia, Gournia, Knossos, Sklavokambos, Phaestos and Hagia Triada) were significantly different from those in the east, yet were all mutually consistent (Table 2, Fig. 2). The same differences and similarities were then confirmed by determination of the palaeointensity of the geomagnetic field (Table 2), although such determinations are less precise. These results were unexpected as it had been thought that the final destruction levels on Crete would have had similar ages. More surprising was the observation that the directions and palaeointensities determined for central Crete were the same as those of the Plinian ashfall deposits on Santorini, while the directions of the sites in eastern Crete were the same as those of the later paroxysmal tephra deposits on Santorini. The central Crete destruction levels are archaeomagnetically synchronous with the Plinian ashfall and there is a time gap before the destructions in eastern Crete and the paroxysmal eruption of Santorini.

Conclusions

The fact that the Akrotiri hearth appears to have been last fired at some time before the destructions in Crete and also before the ashfall on Akrotiri is consistent with the archaeological evidence[36,37] that this Minoan settlement was originally destroyed by an earthquake and that partial clearance had been undertaken before it was covered by ash. However, the scatter in both directions and palaeointensities needs to be improved and this is, anyway, only one structure. The determination of reliable LMIA geomagnetic field parameters, therefore, still require examination of an undoubted LMIA destruction level. While a difference in time between the Akrotiri hearth and the basal Plinian ashes can be considered as strongly indicated, rather than confirmed, there is little doubt about the difference between both the directions and palaeointensities of the Plinian ash/central Crete sites and those of the paroxysmal tephra/eastern Crete destruction levels. However, any evaluation of the actual time represented by the age difference can only be an approximation. If the central Cretan sites and the Thera basal pumice values are combined, giving equal weight to each fired or volcanic level, then their mean direction is 356.1°, 60.0° and the 95% confidence radius is 0.9°. (Knossos is excluded because the only site that was allowed to be sampled was a kiln which may not have been fired at the time of the destruction level—although its similarity in direction and intensity suggests that it was last fired within 2–3 yr of the destruction levels at other central Cretan sites.) Combining Palaikastro, Kato Zakros and the paroxysmal Minoan tephra directions gives a mean direction of 357.1°, 54.8° and a 95% confidence radius of 2.6. The difference in direction is thus 5.2° with the summation of the 95% radii as 3.5° (calculated on a conservative level of analysis as a smaller precision is obtained when these are combined at a sample level). The difference in direction, 1.7–5.2°, is small although clear and confirmed by the palaeointensity differences. However, the rate of secular variation of the geomagnetic field is not known for this time. A few records from Bulgaria[23] span the probable age of these events, but lack of information on the precise age of the Santorini/Cretan events and errors in the ^{14}C dating for this time makes any comparison uncertain. New magnetic studies[25] of the VEMA V10-50 core of sediments from the eastern Mediterranean[5] confirm that a major tephra layer, previously attributed to the Minoan eruption on the basis of refractive indices, also has identical Fe:Ti ratios and Mn, Al, Si and Mg content in its magnetic minerals. The overlying carbonate sediments show a decrease in inclination during this period (Fig. 3) but the rate can only be calculated from sedimentation rates which are themselves only estimates. Thus the change in inclination could be some $0.07° yr^{-1}$, which is slower than the present-day total change in the regional geomagnetic field of some $0.25° yr^{-1}$. The difference in time could be between 100 and 7 yr but the longer time interval seems unlikely, and it is archaeomagnetically unlikely that the time difference could be as low as 7 yr as the observations from the tephra deposits would then show some degree of smearing. A time gap of some 20 ± 10 yr therefore seems to be the most reasonable estimate for this interval.

Implications

The mere existence of a time gap is significant. The fact that it was the central Cretan destruction levels that were archaeomagnetically synchronous with the Plinian ashfall means that the ash itself can have had little direct influence on the Minoan civilization on Crete. The thickness of the ash mantle over Santorini would probably have made it essentially uninhabitable, but only 5 cm of ash is thought to have fallen on Crete[5,6] and this was mostly in the east, where civilization appears to have continued. It seems almost certain, therefore, that the central Cretan destruction levels were caused by an earthquake—the intense burning presumably resulting from oil spills catching fire, and so forth. Knossos seems to be the only central Cretan site in which habitation continued after seismic devastation, which may imply that this area is geologically less vulnerable to earthquakes. Some 10–30 yr later, the violent eruption of Santorini was probably accompanied by tsunamis and air percussion waves. It seems unlikely that tsunamis were the direct cause of the destruction of the remaining Minoan settlements on eastern Crete as extensive fire destruction is inconsistent with such an explanation. If this 'final' event was only a few years after the Plinian ashfall, with associated earthquakes, then weakened buildings are likely to have been flattened by the percussion air wave, but a time interval of 10–30 yr would suggest that this destruction level was also associated with earthquake activity, rather than being directly caused by the

volcanic eruption. Such earthquakes would obviously be related to the increase in volcanic activity but the firing was again probably due to the seismic disturbance. Firing by invading Myceneans[38,39] immediately after the seismic destruction cannot be excluded, although the extent and intensity of burning is comparable with the central Crete destructions which are not considered to be associated with invasion.

This hypothesis for the end of the Minoan civilization has little direct bearing on postulated similarities with the Atlantis legends or with *Exodus*. Plato's descriptions do not obviously refer to volcanic activity, but are consistent with the second eruptive phase, because the end of Atlantis would then relate to the sudden end of the Minoan civilization simultaneously with the disappearance of the island of Santorini as its 80 km^2 caldera formed.

Other implications of this study include the evolution of this form of volcanic activity and the determination of tephra emplacement temperatures. Another implication is the way in which earthquakes near Crete can affect different parts of this island. This seems to be consistent with the evidence for changes in sea-level[40] which also define separate tectonic units. These, and the archaeological implications clearly require study of Minoan destruction levels at other places and times, particularly at Knossos. It would be especially valuable to extend such archaeomagnetic observations to fired levels within the well-dated Egyptian sequences, as well as Mycenae, to determine both the relative and absolute ages of these events.

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Requirement of either of a pair of *ras*-related genes of *Saccharomyces cerevisiae* for spore viability

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Cells of the yeast, Saccharomyces cerevisiae, *containing disruptions of either of two genes that are members of the* ras *oncogene family are viable, but haploid yeast spores carrying disruptions of both genes fail to grow.*

THE cellular proto-oncogenes of the transforming genes from Harvey and Kirsten murine sarcoma virus (c-*ras*^{H} and c-*ras*^{K}, respectively) represent two members of a family of related genes called *ras* (ref. 1). Activated forms of these genes have been isolated from some human cancers and/or cancer-derived cell lines that are able to transform NIH 3T3 cells in a transfection assay. Point mutations at amino acid position 12 or 61 (refs 2–9) have been shown to be necessary and sometimes sufficient for the transforming phenotype[5–7].

The *ras* gene family shows extensive evolutionary conservation[10]. Cellular *ras* genes have been found in a wide variety of vertebrates. The known range of conservation has been extended to *Drosophila melanogaster*[11] and, now, to the yeast *Saccharomyces cerevisiae*[12,13]. Recently, there have been isolated from the yeast genome two DNA fragments that cross-hybridize with a viral *ras* probe[13], while a gene located near the yeast actin and tubulin genes also shares homology with the *ras* gene family[12]. A set of *in vivo*-labelled yeast proteins are specifically immunoprecipitated by anti-*ras* monoclonal antibodies prepared against the mammalian *ras*-encoded protein, p21 (ref. 14).

The DNA sequences of the two *ras*-related DNA fragments isolated by DeFeo-Jones *et al*[13] (c-*ras*^{sc}*1* and c-*ras*^{sc}*2*) have

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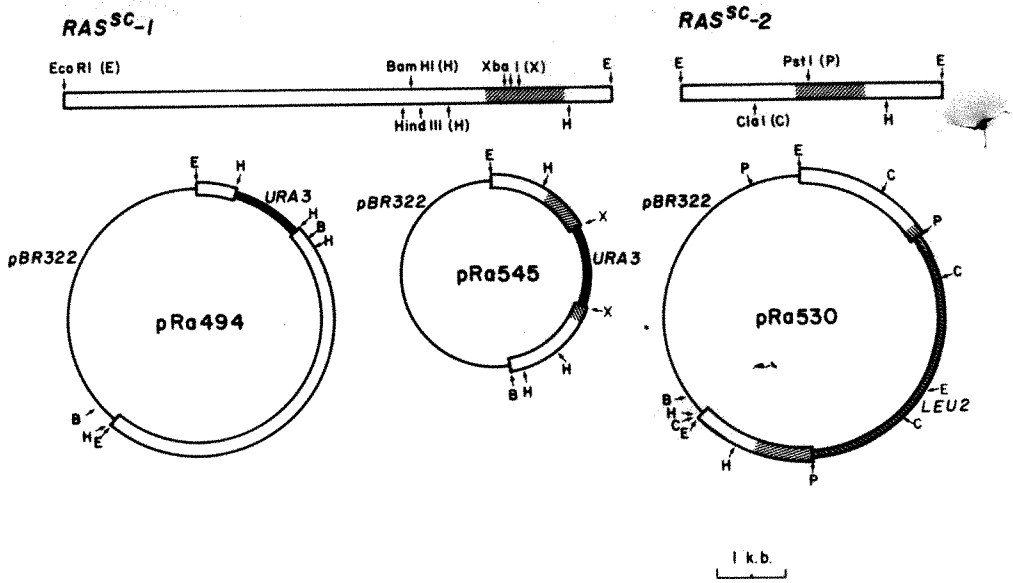


Fig. 1 *Restriction* endonuclease maps of the cloned *RAS*^{sc}*1* and *RAS*^{sc}*2* genes and recombinant plasmids used in the gene disruption experiments. The hatched portions of each map correspond to the location of the yeast *RAS* gene. The plasmids were constructed as follows: pRa545, a 1.1-kb *Hin*dIII fragment containing yeast *URA3* was ligated into the *RAS*^{sc}*1* gene, at the *Xba*I sites; pRa494, the *URA3* gene was substituted for the *Hin*dIII fragment containing *RAS*^{sc}*1* and the *Hin*dIII fragment adjacent to it; pRa530, a 4-kb *Pst*I fragment containing the yeast *LEU2* gene was ligated into the *Pst*I site within the *RAS*^{sc}*2* coding sequence. DNA restriction fragments to be subcloned were gel-purified and ligated using published procedures[34]. The *URA3* gene present in pRA545 and pRa494 and the *LEU2* gene in pRa530 were derived from YEP24 (ref. 35) and YEP13 (ref. 36) respectively. To construct pRa545, the two small *Xba*I fragments present within *RAS*^{sc}*1* were replaced by a *URA3*-containing *Hin*dIII fragment. This was accomplished by filling-in the protruding 5′ ends of the DNA fragments with DNA polymerase I (large fragment) and then flush end-ligating the fragments together. This recreates *Xba*I endonuclease restriction sites at the junctions.

been determined and are presented elsewhere[15,16]. Sequence analysis of the two genes, designated *RAS*^{sc}*1* and *RAS*^{sc}*2*, reveals open reading frames of 309 and 322 amino acids respectively. The putative proteins encoded by these reading frames are highly homologous to each other for the first 180 amino acids, at which point they diverge. Homology is again found at the extreme C-terminal end of each peptide. A striking pattern of amino acid sequence homology is also found when the putative yeast proteins are compared with the mammalian *ras* protein, p21. The NH_2-terminal 170 amino acids of the yeast and mammalian proteins are >60% homologous. The fact that such evolutionarily divergent organisms exhibit such extensive sequence conservation suggests that the *RAS* gene products fulfill an essential role in cell growth and metabolism. Here we show that the two yeast *RAS* genes isolated by D. DeF.-J. *et al.*[13] constitute an essential gene family. At least one functional member of the family is required for spore germination and probably for cell growth.

Viability of yeast strains

We have constructed null mutations in the *RAS* genes to test directly the phenotype of cells lacking these genes. These experiments use yeast transformation techniques which have been developed recently (reviewed in ref. 17). In yeast, introduction of exogenous DNA into the genome requires homology between the recipient chromosome and the donor fragment. This requirement allows one to direct DNA fragments to specific sites within the yeast genome. We have used these transformation systems to integrate disrupted *RAS* genes into the yeast genome, excluding the wild-type genes in the process.

Figure 1 shows the restriction maps of cloned *Eco*RI fragments containing *RAS*^{sc}*1* and *RAS*^{sc}*2*. Part or all of these two restriction fragments have been used to construct a series of recombinant plasmids containing the wild-type *URA3* or *LEU2* gene. Two mutations were constructed in *RAS*^{sc}*1* (Fig. 1). The first, contained in plasmid pRA494, harbours a 1.1-kilobase (kb) *Hin*dIII restriction fragment which encodes *URA3*, substituted for 2.3 kb of *RAS*^{sc}*1* DNA. This deletion lacks the entire *RAS*^{sc}*1* gene plus an additional 1.1 and 0.2 kb of noncoding sequence 5′ and 3′ to *RAS*^{sc}*1*, respectively. The second mutation, in plasmid pRA545, contains the *URA3* gene in place of two small *Xba*I restriction fragments located within the *RAS*^{sc}*1* open reading frame (Fig. 1). This deletion spans amino acids 63–150 of the putative *RAS*^{sc}*1* coding sequence. A single disruption of *RAS*^{sc}*2* was constructed in plasmid pRa530 by inserting a 3.8-kb *Pst*I restriction fragment, encoding *LEU2*, into a *Pst*I site that corresponds to amino acid 65 in the *RAS*^{sc}*2* open reading frame. These insertions should therefore disrupt the *RAS*^{sc} gene products.

Plasmids pRa494, pRa530 and pRa545 were digested with the appropriate restriction endonucleases to generate DNA fragments containing *RAS* DNA flanking the *LEU2* and *URA3* insertions. These conditions lead to replacement of the wild-type locus with the transforming sequence as outlined in Fig. 2 (ref. 18). As it is possible that the *RAS* genes encode essential functions, the restricted plasmids were introduced into a diploid strain homozygous for *ura3* and *leu2* (TX2).

To identify those transformants having the desired molecular structure, genomic DNA was prepared from pRa494, pRa530 and pRa545 transformants and analysed by the method of Southern[19] (Fig. 3*a*). Most of the transformants give hybridization patterns consistent with the simple replacement in one chromosome homologue of the wild-type locus by the null or the disruption mutation. *RAS*^{sc}*1* is contained on a 2.9-kb *Bam*HI–*Eco*RI restriction fragment. In the pRa545-derived mutations this fragment expands to 3.8 kb, due to the insertion of the *URA3* gene. In the pRa494 mutation the fragment size decreases to 1.8 kb, due to the deletion of the entire *RAS*^{sc}*1* locus. These structures are shown in Fig. 3*b*, together with analogous diagrams for the wild-type and mutant *RAS*^{sc}*2* genes. The hybridization data for transformants of pRa545, pRa530 and pRa494 (Fig. 3*a*) are consistent with the structures outlined in Fig. 3*b*. In each case, the transformants contain a wild-type *RAS*^{sc}*1* allele on one chromosome and the null or disrupted allele on the other.

The phenotypes of haploid yeast strains lacking a functional copy of either *RAS*^{sc}*1* or *RAS*^{sc}*2* were determined in haploid meiotic segregants derived from the transformants. The meiotic cell cycle in yeast is initiated when diploid strains are placed on a nitrogen-deficient medium containing a nonfermentable carbon source. The end product of each meiotic event is four haploid spores, collectively called a tetrad. As the *ras* mutations are heterozygous in the diploid transformants, two spores in every tetrad will contain the mutant *ras* allele and two will contain the wild-type allele. The *ras*^{sc}*1* mutations are associated with *URA3* and the *ras*^{sc}*2* mutation is associated with *LEU2*, so that Ura$^+$ and Leu$^+$ haploid spore clones mark those haploid segregants that lack a functional copy of *RAS*^{sc}*1* or *RAS*^{sc}*2*

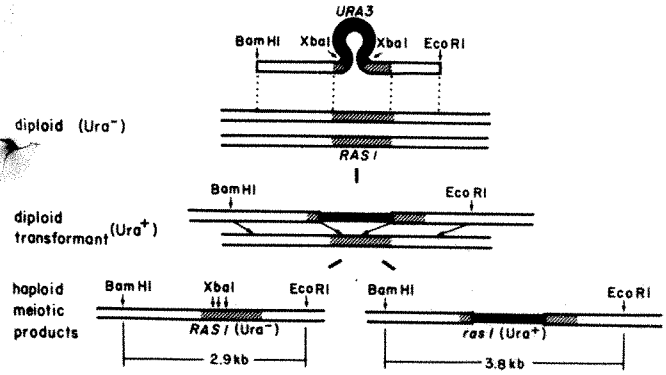


Fig. 2 Schematic drawing of the transformation of diploid strain TX2 with the *Eco*RI–*Bam*HI *RAS*^{sc}*1* fragment of pRa545. The linear fragment integrates into the yeast chromosome at *RAS*^{sc}*1*, giving rise to a heterozygous mutation in *RAS*^{sc}*1*. Sporulation of this diploid gives rise to haploid spores containing either the wild-type or mutant *RAS*^{sc}*1* locus. Strain TX2 was transformed with linear restriction fragments by the method of Beggs[37]. Gene disruptions 494 and 530 were created in a similar manner by transforming TX2 with *Eco*RI-restricted pRa-494 or *Xba–Hin*dIII-restricted pRa530 and selecting for uracil or leucine prototrophy, respectively.

respectively; this is outlined schematically in Fig. 2. The recovery of Ura$^+$ and Leu$^+$ segregants from TX2-545 and TX2-530, respectively, indicates that the *ras*^{sc}*1-545* and *ras*^{sc}*2-530* mutations do not result in cell inviability. The Ura$^+$ and Leu$^+$ phenotypes segregate normally through meiosis and Ura$^+$ or Leu$^+$ spores have no apparent growth disadvantage on the rich medium used for the analysis. It seems, therefore, that neither *RAS*^{sc}*1* nor *RAS*^{sc}*2* is separately essential for ascospore germination and growth. Southern analyses of tetrads from TX2-545 or TX2-530 (Fig. 3*a*) show that the Ura$^+$ and Leu$^+$ spore clones contain only the mutant copy of *RAS*^{sc}*1* or *RAS*^{sc}*2*, respectively.

The fact that both *RAS* genes are not required for vegetative growth (mitotic cycle) does not exclude the possibility that both are required for meiosis. To test this, we have constructed diploid strains homozygous for either the *ras*^{sc}*1-545* or *ras*^{sc}*2-530* allele. In both cases these diploids sporulated normally, giving rise to a high proportion of asci with four spores. This indicates that meiosis is normal when the products of one or other of the genes *RAS*^{sc}*1* and *RAS*^{sc}*2* are absent.

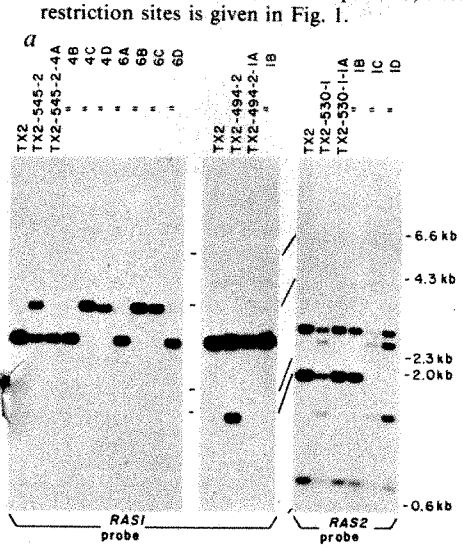
Unlike TX2-545 and TX2-530, transformants derived from pRa494 are heterozygous for a lethal mutation, as only two viable spores in each tetrad of TX2-494 are recovered. Those spores containing the deletion, marked by *URA3*, do germinate, but undergo only one to three rounds of cell division. The fact that the deletion in TX2-494 includes >1 kb of DNA upstream of the *RAS*^{sc}*1* gene, and the observation that internal disruptions in the *RAS*^{sc}*1* and *RAS*^{sc}*2* open reading frames are not lethal, suggests that some essential function separate from *RAS*^{sc}*1* lies at least partially within the *ras*^{sc}*1-494* deletion.

Spore viability

Our results suggest that neither *RAS*^{sc}*1* nor *RAS*^{sc}*2* separately has an essential function, since no phenotypic change is associated with haploid strains lacking either a functional *RAS*^{sc}*1* or *RAS*^{sc}*2* gene. Neither gene alone is absolutely required for either the mitotic or the meiotic cell cycle. But it is possible that the two *RAS* genes may code for proteins with overlapping but not necessarily identical functions. To test this possibility, we constructed a diploid, EG53, that is heterozygous for both the *RAS*^{sc}*1* and *RAS*^{sc}*2* disruptions by mating a Ura$^+$ haploid segregant from TX2-545 (*RAS*^{sc}*1* disruption) with a Leu$^+$ haploid segregant from TX2-530 (*RAS*^{sc}*2* disruption). Tetrad analysis was performed on the resultant meiotic progeny.

Because *RAS*^{sc}*1* and *RAS*^{sc}*2* are unlinked (see below), the two genes should segregate independently in meiosis, and give rise to recombinant spores (Ura$^+$, Leu$^+$ and Ura$^-$, Leu$^-$) frequently. However, no Ura$^+$ Leu$^+$ recombinants were recovered in 60 tetrads analysed, although Ura$^-$, Leu$^-$ segregants were frequently observed. Moreover, as shown in Table 1, a peculiar pattern of spore germination was observed in the dissected tetrads. Relatively few tetrads with four viable spores were recovered (7/60), whereas ascopore germination in related crosses was good (Table 1). These seven tetrads are parental with respect to *URA3* and *LEU2* in the sense that none of the spores was recombinant. Similarly, many more asci with three viable spores per tetrad are recovered in EG53 (30/60) relative

Fig. 3 *a*, Southern blot analysis of gene disruptions in *RAS*^{sc}*1* and *RAS*^{sc}*2*. The 10 lanes in the first panel correspond to genomic DNA from parent (TX2), heterozygous transformant TX2-545-2 and haploid spore clones from two tetrads of TX2–545-2. Haploid spore clones 4A, 4B, 6A and 6D were Ura$^-$, while spore clones 4C, 4D, 6B and 6C were Ura$^+$. The four lanes in the second panel correspond to DNA from parent, transformant 494-1, and two haploid spore clones from a tetrad of TX2-494-1. Only haploid spores containing the wild type *RAS*^{sc}*1* gene grow into colonies. DNA in the first two panels was digested with *Bam*HI and *Eco*RI and probed with nick-translated *RAS*^{sc}*1* DNA (*Eco*RI–*Bam*HI fragment). In the third panel the six lanes correspond to DNA from the parent, pRa530 transformant (TX2-530-1), and spore clones from a tetrad of the transformant. DNA was restricted with *Cla*I and *Hin*dIII and probed with *RAS*^{sc}*2* nick-translated DNA (*Eco*RI fragment). Spore clones 1A and 1B are Leu$^-$ while spore clones 1C and 1D are Leu$^+$. The migration positions of the phage λ-*Hin*dIII restriction fragment are indicated to the left of the third panel. *b*, Restriction endonuclease maps of *RAS*^{sc}*1*, *RAS*^{sc}*2* and gene disruption present in *a*. The key to the restriction sites is given in Fig. 1.



Methods: Genomic yeast DNA was isolated using a published procedure[38], restricted with the appropriate restriction enzymes, electrophoresed through 0.7 agarose in Tris-borate EDTA buffer[39], and blotted onto nitrocellulose[40]. The nitrocellulose was hybridized with ^{32}P-labelled nick-translated DNA in a solution of 6× SCP (1× SCP = 0.1 M sodium chloride, 0.03 M sodium phosphate, 0.001 M EDTA, *p*H 6.2), 0.1% *N*-lauroyl sarcosine, 0.06 mg ml^{-1} sonicated calf thymus DNA for 16–24 h at 65 °C. The filter was washed sequentially twice for 10 min each at 60 °C in 2× SCP, 0.01% SDS and 2× SCP. Autoradiographs of filters were made using Kodak X-omat R film.

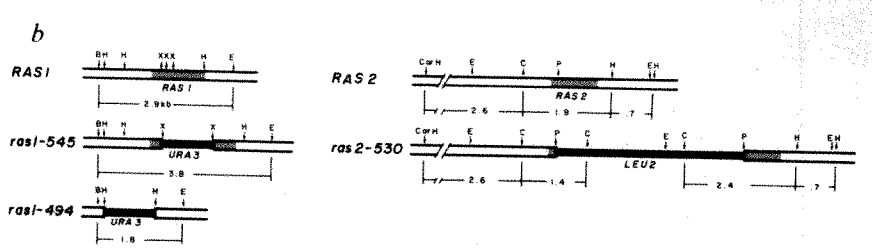


Table 1 Lethal segregation patterns of *RAS* disruptions

Disrupted *RAS* gene	Strain	Viable spores per tetrad: 4	3	2	1	0	% Germination of spores with *RAS* disruptions
—	TX2	27	3	0	0	0	
RAS1	TX2-545-2	24	2	1	0	0	48 (Ura^+ spore clones)
RAS1	TX-545-3	6	0	0	0	0	50 (Ura^+ spore clones)
RAS2	TX2-530-1	16	5	3	1	0	48 (Leu^+ spore clones)
RAS2	TX2-530-2	4	2	1	0	0	50 (Leu^+ spore clones)
RAS1	TX2-494-2	0	0	65	11	4	0 (Ura^+ spore clones)
RAS1, RAS2	EG53	7 (7PD)	30 (29T)	20 (13NPD)	3	0	0 (Ura^+, Leu^+ spore clones)

Spore viability of diploid strains heterozygous for *ras* disruptions. EG53, heterozygous for *RAS1* and *RAS2* disruptions, was constructed by crossing Ura^+ and Leu^+ haploid segregants from TX2-545 and TX2-530 respectively. In EG53 all asci containing four viable spores were parental ditype (PD) for Leu^+ and Ura^+, 29/30 asci containing three viable spores were tetratype (T) and 13/20 asci containing two viable spores were non-parental ditype (NPD). The seven asci with two viable spores that are not non-parental ditype are the result of nonspecific spore lethality.

to the related crosses. In most of these tetrads (29/30), *URA3* and *LEU2* are in the tetratype configuration (one of each parental and one of each recombinant type). By inference, the inviable spore is always the Ura^+, Leu^+ recombinant. There is also a disproportionately high number of tetrads with only two viable spores (20/60), and most of these asci (13/20) are non-parental (all of the spores are recombinant) with respect to *URA3* and *LEU2*. Again the inviable spores in these 13 tetrads are inferred to be those with both disrupted *RAS* genes (the Ura^+, Leu^+ spores). The results of this analysis demonstrate a striking correlation between the observed lethal segregation pattern and the segregation of the two mutant *RAS* genes, and provide strong evidence that the double mutant (Ura^+, Leu^+) causes lethality in haploids. These data, therefore, indicate that the *RAS^{sc}1* and *RAS^{sc}2* genes encode an essential function.

Visual inspection of the inviable spores reveals that not only do they fail to undergo a single round of cell division, but they also fail to form buds. Since bud emergence in vegetatively growing cells and germinating spores occurs early in the S phase of the cell cycle[20], this observation suggests that the inviable spores may be blocked quite early in the cell division cycle. The inviability, however, does not distinguish between a function for *RAS* that is specifically related to the cell cycle *per se* as opposed to a general role in cell growth and/or spore germination. Note also that this lethal spore phenotype is quite different from that observed for the lethal segregants of TX2-494. In the latter case, the spores were able to germinate and undergo one to three rounds of cell division before mitotic growth ceased. This phenotypic difference further supports the notion that the lethal mutation in TX2-494 is not in the *RAS^{sc}1* gene.

RAS^{sc}1 is located on chromosome XV

During the genetic analysis of the *RAS^{sc}1* mutants, we observed genetic linkage between *RAS^{sc}1* and *ade2*, 13 recombination units, on the right arm of chromosome XV. Additional mapping data between *pet17* and the mutant *ras^{sc}1* locus place the *RAS^{sc}1* centromere proximal to *ADE2* (see Table 2). A temperature-sensitive (ts) lethal mutation *tsm8740*, has recently been mapped to chromosome XV, 13 centimorgans from *ade2* (ref. 21).

Table 2 Mapping data for *RAS1* and strain list

Strain	Map interval	Ascus type: PD	NPD	T	Map distance (centimorgans)
TX2-494-2	Lethal-*ade2*	34	0	12	12.4
DCX103	Lethal-*ade2*	53	0	22	14.7
DCX103	Lethal-*ras^{sc}1-494*(Ura^+)	75	0	0	< 0.7
DCX102	Lethal-*pet17*	26	2	35	37.3
DCX102	Lethal-*ras^{sc}1-494*(Ura^+)	63	0	0	< 0.8

Strain	Genotype	Ref.
TX2	$\frac{a\ leu2\ ura3\ can1\text{-}100\ ade2\text{-}1\ his3\ trp1\ +\ lys1}{\alpha\ leu2\ ura3\ can1\text{-}101\ +\ +\ trp1\ lys1\ +}$	This work
K381-9D	*α spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1*	30
K392-19D	*α spo11 ura3 can1 cyh2 ade2 his7 hom3 tyr1*	30
Be326	*a tsm8740*	21
EG45–7D	*a tsm8740 ade2-1 ura3 his3*	This work
DCX102	$\frac{a\ leu2\ ura3\ can1\ +\ +\ +\ +\ +\ +\ +\ +\ +\ ras^{sc}1\text{-}494}{\alpha\ +\ ura3\ +\ ade6\ arg4\ spo11\ aro7\ asp5\ met14\ lys2\ trp1\ pet17\ +}$	This work
DCX103	$\frac{a\ leu2\ +\ ura3\ can1\ +\ +\ +\ +\ ras^{sc}1\text{-}545}{\alpha\ +\ hom3\ ura3\ can1\ cyh2\ tyr7\ his7\ ade2\ +}$	This work
EG53	$\frac{a\ leu2\ ura3\ can1\ trp1\ +\ +\ ade2\ ras^{sc}1\text{-}545\ +}{\alpha\ leu2\ ura3\ can1\ trp1\ lys1\ lys2\ +\ +\ ras^{sc}2\text{-}530}$	This work

Results of tetrad analysis on strains containing the *ras^{sc}1-494* allele. Genetic procedures are standard[31] and the sporulation plate media are described elsewhere[32]. DCX102 and DCX103 were derived from mating haploid segregants of a *ras^{sc}1-494* strain to K381-9D and K382-19D immediately after dissection. This lethal rescue technique is described elsewhere[33]. The *tsm8740* allele in EG45-7D was derived from Be326.

Although we find that this mutation is tightly linked to *RAS*^{sc}*1*, less than 1 map unit distant (unpublished observations), it is not allelic to *RAS*^{sc}*1*. This has been determined by mating a haploid strain bearing *tsm8740* (EG45-70) with a haploid containing the large *RAS*^{sc}*1* deletion from TX2-494. (Although spores containing the *ras*^{sc}*1-494* deletion fail to grow into colonies, they can be mated immediately after germination.) The resulting diploid strain, doubly heterozygous for *tsm8740* and the *ras*^{sc}*1-494*, is not temperature-sensitive for growth, indicating that *tsm8740* is complemented by the wild-type allele on the ras^{sc}*1-494* chromosome. Similar mapping experiments indicate that *RAS*^{sc}*2* is unlinked to *ADE2* and *RAS*^{sc}*1*; its precise location has not yet been determined.

Conclusions

The data presented here demonstrate that *RAS*^{sc}*1* and *RAS*^{sc}*2* encode an essential function in yeast. The viability of yeast cells containing disruptions of either of these genes indicates that the function(s) specified by either is sufficient to support both mitotic and meiotic cell division cycles, but that at least one wild-type gene is required for viability. There is no apparent correlation between the rates of spore germination or growth and the disruption of either *RAS* gene.

The inability to recover ascosporal clones that contain both mutant *RAS* genes indicates that the two genes encode proteins with similar if not identical functions. Our results do not exclude the possibility that *RAS*^{sc}*1* and *RAS*^{sc}*2* have separate functions as we have observed growth rates only under a limited set of conditions. However, these results do show that the *ras*-related sequence identified by Gallwitz and colleagues[12] does not specify the same function as *RAS*^{sc}*1* and *RAS*^{sc}*2*, as the sequence fails to complement the defect of the double *RAS* mutant. A situation in which two unlinked genes have nearly identical functions exists for several other yeast genes. Histones H2A (ref. 22) and H2B (ref. 23), and the ribosomal protein p51 (ref. 24) are encoded by two unlinked genes. Disruption of only one of the gene pair generally causes no phenotypic change while disruption of both genes has severe consequences on cell viability. Although untested, it is likely that histones H3 and H4 (ref. 25), glyceraldehyde 3-phosphate dehydrogenase[26], and several other ribosomal proteins which have two structural genes[27] may fall into this category of duplicated essential genes.

The results obtained from the analysis of one of the *RAS*^{sc}*1* mutations, *ras*^{sc}*1-494*, suggests that another essential cellular function is tightly linked to *RAS*^{sc}*1*. That this essential gene is not *RAS*^{sc}*1* is suggested by several lines of evidence. First, the deletion defined by this mutation extends 1.1 kb 5′ and 0.2 kb 3′ beyond the *RAS*^{sc}*1* coding sequence, and therefore includes more than just *RAS* DNA. Second, an internal *RAS*^{sc}*1* deletion in the highly conserved region of amino acid homology does not by itself result in cell lethality. Spore inviability is observed only in cells that carry this internal mutation in conjunction with the *ras*^{sc}*2* disruption. Third, the lethal phenotype of *ras*^{sc}*1-494* spores is quite different from that of the *ras*^{sc}*1 ras*^{sc}*2* double mutants. Spores containing *ras*^{sc}*1-494* germinate and undergo one to three rounds of cell division before growth is arrested, whereas *ras*^{sc}*1 ras*^{sc}*2* spores fail to divide or initiate bud formation. Although these data do not exclude other explanations, the most likely interpretation is that another essential gene resides in close proximity to *RAS*^{sc}*1*.

Comparison of the presumed amino acid sequences of *RAS*^{sc}*1* and *RAS*^{sc}*2* reveals a very large region of non-homology that is flanked by highly homologous amino acid sequences. The NH_2-terminal 180 amino acids are 83% conserved but the next 100 amino acids show only 20% conservation[15,16]. The proteins again show a high degree of homology at their C-terminal nine amino acids. Despite this large divergent region, however, the two gene products complement each other. This lack of selective pressure can be accounted for if the diverged portions of the *RAS* polypeptides are removed proteolytically, an event that is known to occur in the C-terminal region of the mammalian *ras* genes[28,29]. Alternatively, these portions of the yeast proteins may encode a function not particularly dependent on primary sequence. It may be noteworthy that the divergent region of each putative *RAS* polypeptide does show a similar amino acid composition.

Judging from the extreme evolutionary conservation of the *ras* genes, it is not surprising to find that their products have a vital role in cell growth or metabolism in yeast. What does this analysis suggest this role might be? The failure of *ras*^{sc}*1 ras*^{sc}*2* double mutants either to germinate or to form buds suggests that the *RAS* gene products are required early in the cell division cycle. These data are also consistent with the proteins being quite rare and/or highly susceptible to proteolysis during the sporulation process. More extensive knowledge about *RAS* function(s) awaits the isolation and analysis of conditional mutations in *RAS*.

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Far-UV and extreme-UV observations of SS Cyg and U Gem from Voyagers 1 and 2

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Recent studies of dwarf novae in the UV and X-ray regions have suggested that much of the energy produced in a dwarf nova is emitted in the far-UV and extreme-UV regions (100–1,200 Å). We report here on observations of SS Cyg and U Gem obtained with the Voyager UV spectrometers (UVS) in the 500–1,200 Å region. SS Cyg has been detected in quiescence at a flux level of 9×10^{-13} erg cm^{-2} s^{-1} Å^{-1} at 1,000 Å. U Gem was not detected in quiescence. No emission shortward of the Lyman limit (912 Å) is detected in either star. Observational upper limits to the extreme-UV flux in the 540–740 Å band from SS Cyg and U Gem correspond to 2×10^{-13} erg cm^{-2} s^{-1} Å^{-1}. A far-UV (λ_{eff} = 1,050 Å) light curve for an outburst in SS Cyg is presented. The slope of the far-UV flux distribution during the rise to maximum is observed to be correlated with the UV brightness. The nature of this correlation supports the suggestion that the outburst begins at the outer edge of the accretion disk and moves inward. The far-UV flux distributions of SS Cyg and U Gem in outburst were found to be remarkably similar, both exhibit a substantially flatter ($F_\lambda \propto \lambda^{-0.5}$) distribution than that observed in the IUE–UV ($F_\lambda \propto \lambda^{-2.3}$). This result is contrary to current disk models which predict a continued rise in flux in the far-UV. Evidence for a wind in the Lyman lines of hydrogen is seen in SS Cyg. No evidence of a wind was observed in U Gem.

The UVS[1] on board Voyagers 1 and 2 are nearly identical objective grating instruments having maximum sensitivity in the 500–1,200 Å region and a dispersion of 9.26 Å per detector channel (resolution ~20 Å). Previous stellar observations and the absolute calibration have been discussed by Holberg *et al.*[2]. A decline in detector sensitivity towards longer wavelengths establishes ~1,200 Å as a practical long wavelength limit for observing sources of the brightness of SS Cyg and U Gem. We have therefore restricted analysis of Voyager data to wavelengths shortward of 1,175 Å.

SS Cyg has been observed with the UVS during two outbursts and U Gem during one outburst. Table 1 lists the dates of observation and the characteristics of these outbursts. In quiescence, SS Cyg has been detected at the 2σ level in the 1,000 Å region at a flux level of 9×10^{-13} erg cm^{-2} s^{-1} Å^{-1}. U Gem has not been detected in quiescence with the Voyager UVS. During the intervals listed in Table 1, the Voyager scan platform was fixed in the direction of the target, while the spacecraft limit cycle motions allowed the field of view of the spectrometer to wander on and off the target to obtain both source and background signals. Except for the investigation of the change in the slope of the flux distribution in SS Cyg during the rise to maximum on JD 2444373, only on-axis (±0.02°) source spectra are discussed here. For that investigation marginally off-axis (±0.04°) spectra were combined with adjacent (in time) on-axis spectra. Tests of UVS spectra show that the relative distribution within a spectrum is unaffected by it being obtained off-axis. Thus, this technique results in an improved signal-to-noise ratio within the spectrum at the expense of absolute photometry.

The far-UV light curve of the first outburst (JD 2444373) in SS Cyg is presented in Fig. 1. The light curve was generated by averaging the 925–1,175 Å flux for on-axis spectra. Whenever possible, spectra closely spaced in time were combined. During the rise to maximum this time 'window' was set at 1 h. For the remainder of the outburst, time bins up to 12 h were used. Also plotted in Fig. 1 is the ratio of the flux in the long wavelength channels (1,090 ± 40 Å) to the short wavelength channels (960 ± 40), Lyβ is excluded from both bands. The second outburst observed with the UVS exhibited no significant change in flux throughout the 4 days of visual light curve. A mean 1,050 Å flux of $3.00\pm0.10\times10^{-11}$ erg cm^{-2} s^{-1} Å^{-1} was measured with a $F_{950}/F_{1,090}$ ratio of 0.97 ± 0.05. The observations of U Gem during maximum also showed no significant change during the 5 days of observation. The mean 1,050-Å flux and $F_{950}/F_{1,090}$ ratio were respectively $1.42\pm0.10\times10^{-11}$ erg cm^{-2} s^{-1} Å^{-1} and 1,03 ± 0.05. The U Gem data were also explored for variability coupled to the orbital period of 4.25 h (ref. 3). No variations in flux >5% were detected.

The far-UV light curve is similar in shape to the observed visual light curve (J. A. Mattei, personal communication)[4]. The rise in the far-UV seems to be delayed by 0.5–1.0 days with respect to the rise in the visual. Large uncertainties in the times of the UV and visual rise to maximum preclude any discussion of the possible delay. However, it is clear that in the earliest stages of the outburst the rise in flux at shorter UV wavelengths (960 Å) is delayed relative to that at longer UV wavelengths (1,090 Å). This is consistent with the observations of VW Hyi by Hassall *et al.*[4] and strongly supports their suggestion that the outburst is initiated at the outer edge of the disk and moves inward. During the decline to quiescence there is a suggestion in the Voyager data that the drop at 960 is more rapid than that at 1,090 Å. The difference is less pronounced than that observed during the rise and will require further observation to confirm its existence.

Figure 2 shows composite outburst flux distributions for SS Cyg and U Gem. The far-UV data for SS Cyg are from the

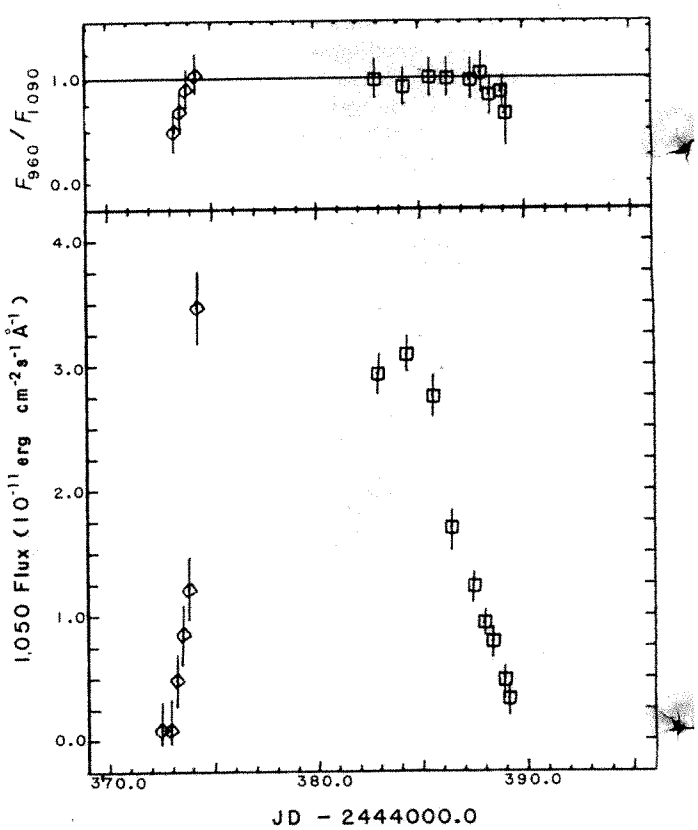


Fig. 1 Far-UV light curve and hardness curve for SS Cyg. Error bars indicate $\pm2\sigma$. ◇, Voyager 1; □, Voyager 2.

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Data ..... Firma .....

second outburst observed with the Voyager 1 UVS (JD 2445272). These data pertain to SS Cyg at maximum light and represent a sum of on-axis spectra having a total integration time of 17,280 s. The IUE data for SS Cyg are from A. V. Holm (personal communication) and were obtained on 14–15 June 1978. The ground-based data are from Oke and Wade[5], obtained on 11 July 1977. All three observations are from long duration outbursts, however, as they are from different outbursts, small adjustments in flux had to be made to get the three segments to match at the overlap points. These adjustments in log F_λ were +0.05 and −0.05 for the IUE and ground-based data respectively. The Voyager far-UV data for U Gem also represent a sum of maximum light, on-axis spectra, with a total integration time of 23,040 s. The IUE data are from Panek and Holm[6] obtained on 10 October 1980. Although representing different outbursts, no adjustments were made between the Voyager and IUE data.

Examination of the Lyman continuum region ($\lambda < 912$ Å) of SS Cyg and U Gem shows no evidence of any extreme-UV emission. Specifically, in the 540–740 Å band Voyager observations establish a 3σ upper limit on the mean flux of 2×10^{-13} erg cm^{-2} s^{-1} Å^{-1} for both stars (see Fig. 2). In the case of SS Cyg such a limit is not entirely unanticipated, as interstellar neutral hydrogen column densities of the order of 10^{20} cm^{-2} have been deduced from soft X-ray observations[7,8]. Our upper limit for the U Gem outburst is, however, of more interest as a considerably lower H I column density has been inferred for U Gem[8]. We can use our result to establish a lower limit on the H I column density in the direction of U Gem. IUE observations of U Gem[6] during quiescence have established that shortward of 2,000 Å the energy distribution of U Gem approximates that of a 30,000 K white dwarf. Such an object will contribute little to the total extreme-UV flux at 640 Å, thus any flux at these wavelengths arises from the region producing the quiescent soft X-rays. Extrapolation of this soft X-ray distribution into the extreme-UV region is very uncertain. Depending on the extrapolation used, neutral hydrogen column densities in excess of 10^{17} to 10^{18} cm^{-2} will be sufficient to reduce the expected extreme-UV flux below our limit of detection. Soft X-ray fluxes, however, are observed to increase dramatically during the outburst phase[8] so that the extreme-UV flux can also be expected to show a corresponding increase. In such conditions, somewhat larger hydrogen column densities would be necessary to satisfy our observed EUV upper limit.

Table 1 Outburst observations of SS Cyg and U Gem

Star	JD of UVS observation (2440000.0+)	Voyager spacecraft and data rate*	JD of visual rise to maximum† (2440000+)	$m_{v_{max}}$†	Approximate duration of outburst† (days)
SS Cyg	4,372.4–4,374.0	1,L	4373	8.4	17
	4,382.7–4,389.0	2,L,H			
SS Cyg	5,278.5–5,282.6	1, L	5272	8.4	18
U Gem	5,397.7–5,402.4	1, L	5393	9.1	17

* 1 = Voyager 1; 2 = Voyager 2. L, 1 spectrum/576 s; H, 1 spectrum/3.84 s.
† From AAVSO (American Association of Variable Star Observers) light curve charts supplied by Mattei (personal communication).

A comparison of the relative far-UV flux distributions of SS Cyg and U Gem indicate that except for strong hydrogen Lyman series absorption in SS Cyg, the two distributions are statistically indistinguishable. If the UVS far-UV distribution is to be represented by a power law of the form $F_\lambda \propto \lambda^\alpha$, α must be -0.5 ± 0.5. This is significantly different from the exponent, ~ -2.3, measured in near-UV (IUE) region. The shape of the distribution does not, however, suggest a power law. Both stars show a noticeable change in slope near 1,000 Å. Note that the change in slopes seen in Fig. 2 is not an artefact of the Voyager flux calibration. Examination of sources having true power law spectra, such as some very hot O subdwarfs and central stars of planetary nebula, shows that Voyager fluxes produce no discontinuity or change of slope in the power laws which characterize IUE and ground-based fluxes. The most likely cause of this change in slope is line blocking in the dwarf nova disk. A comparison of these flux distributions with those of other objects observed with the Voyager UVS indicate that they are very similar in slope and shape to intermediate temperature (~50,000 K) subdwarfs or white dwarfs. In particular, this intercomparison of flux distributions reveals that any object with a suggested effective temperature in excess of ~70,000 K yielded a much steeper slope in the far-UV than either SS Cyg or U Gem. Because SS Cyg and U Gem are at radically different inclinations[9] (38° and 67° respectively) it is unlikely that this absence of evidence for higher temperatures ($T \gtrsim 50{,}000$ K) could be due

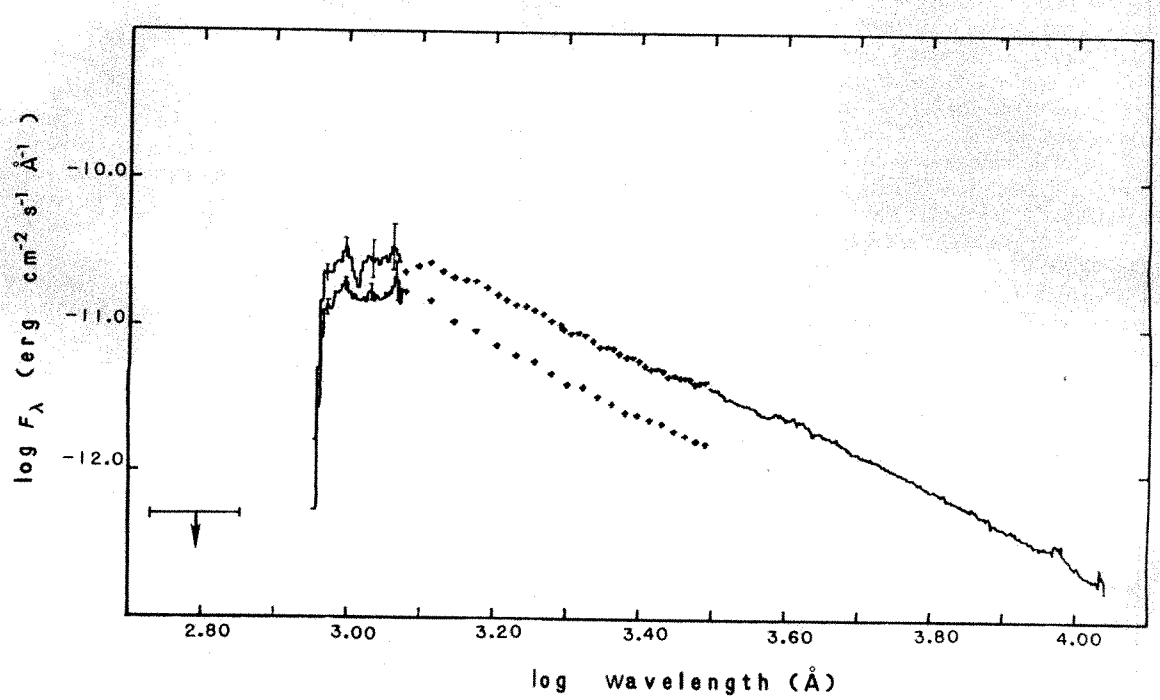


Fig. 2 Composite energy distributions for SS Cyg (upper) and U Gem (lower). The Voyager data (histogram, $\pm 2\sigma$ error bars indicated) are from JD 2445278-83 (SS Cyg) and JD 2445398–402 (U Gem). The IUE data (crosses) are from JD 2443673/74 (SS Cyg, A. V. Holm, personal communication) and JD 2444522 (U Gem[6]). The ground-based data for SS Cyg (solid line) is from JD 2443335 (ref. 5).

to occultation effects. Thus, the inner disk in these dwarf novae must be radically different in structure from that predicted by theory (see ref. 10). Such a change in slope could be due to a breakdown of the thin disk approximation. If the disk became thick it would radiate as a rapidly rotating star[10]. This would satisfy the observed far-UV energy distribution (Fig. 2) and could explain the absence of evidence for a boundary layer through occultation effects and/or reprocessing of radiation.

With the outburst flux distribution for SS Cyg (Fig. 2) a bolometric luminosity can be computed. The shape of the far-UV distribution suggests that only a small amount of energy ($\lesssim 15\%$) is intrinsically emitted shortward of 912 Å. We find that the luminosity for SS Cyg in outburst is $1.3 \pm 0.3 \times 10^{34}$ erg s^{-1} ($\sim 3.3\ L_{\odot}$), assuming a distance of 50 pc (ref. 11). Similar arguments can be made for U Gem. However, the absence of adequate ground-based data makes the resultant luminosity very uncertain. Assuming a 3,000–10,000 Å distribution similar to that of SS Cyg, a luminosity of $1.5 \pm 0.5 \times 10^{34}$ erg s^{-1} is derived for a distance of 75 pc (ref. 12).

As discussed earlier, the only difference between the SS Cyg and U Gem far-UV flux distributions is the presence of Lyman series hydrogen absorption in SS Cyg. [Note neither SS Cyg nor U Gem show any suggestion of He II (specifically 1,085 Å) absorption.] The identification of this line as Lyβ and not O VI is based on the results of a division of the SS Cyg distribution by that of U Gem. This division revealed additional weak absorptions at Lyγ and Lyδ. The data from the earlier outburst observed in SS Cyg with Voyager also show Lyβ absorption. Although in this outburst the line is quite variable in strength. We postulate that most of the observed hydrogen absorption in SS Cyg originates in a wind. Such winds have been observed in other dwarf novae of low inclination[13]. The absence of wind lines in U Gem is also consistent with this model. Interstellar Lyβ is also a likely contributor to the absorption in SS Cyg. However, the observed variability of the feature suggests that it cannot be the principle source of the line.

The principal results from the Voyager UVS investigation of SS Cyg and U Gem in outburst can be summarized as follows. The far-UV light curve confirms the earlier observation of a delay in the rise of flux at shorter wavelengths relative to that occurring at longer wavelengths in the early stages of an outburst. The flux distribution in the far-UV is significantly flatter ($F_{\lambda} \propto \lambda^{-0.5 \pm 0.5}$) than that in the near-UV (IUE) region. No extreme-UV emission was detected in either SS Cyg or U Gem and the shape of their far-UV flux distributions suggest that little intrinsic extreme-UV flux is emitted. A bolometric luminosity of $1.3 \pm 0.3 \times 10^{34}$ erg s^{-1} is derived for SS Cyg in outburst ($d = 50$ pc). A similar luminosity, $1.5 \pm 0.5 \times 10^{34}$ ergs s^{-1}, is deduced for U Gem ($d = 75$ pc). No evidence is found for any significant area of the inner disk/boundary layer region with a temperature in excess of ~50,000 K. The far-UV flux distribution can be best represented by a high gravity ($\log g \sim 6$–8) intermediate temperature ($T_{\rm eff} \sim 50{,}000$ K) stellar atmosphere. These results argue for a significantly different inner disk/boundary layer structure than current theory predicts. Further observations of cataclysmic variables, simultaneous with observations for IUE and ground-based telescopes, are planned.

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Upper limit on solar interior rotation

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The power spectrum of solar total irradiance (flux) variations from the Active Cavity Radiometer Irradiance Monitor (ACRIM) on the Solar Maximum Mission (SMM) spacecraft shows individual 5-min *p*-mode oscillations[1,2] of spherical harmonic degree $l = 0$–2 and radial order $n = 16$–26. An *m*-state splitting analysis based on the widths of the (n, l) multiplets in the spectrum of ACRIM data yields a mean (sidereal) interior rotation rate between 0 and 2.2 times the observed 0.456-μHz equatorial surface rate, consistent with the rapid rotation rate originally claimed by Claverie *et al.*[3] based on a (controversial) splitting interpretation of the these 5-min modes seen in line-of-sight velocity. Rotationally split *p*- and *g*- and *f*-modes have been identified in the temporal power spectrum of the limb-darkening data of Bos and Hill[4], and from these splittings two internal rotation curves[5,6] have been deduced which imply a solar gravitational quadrupole moment J_2 large enough to spoil the precise agreement between general relativity and observations of planetary motion. The splitting of the low-l 5-min *p*-modes implied by these curves is inconsistent with the upper limit derived here, and the reported conflict with einsteinian theory, is therefore, premature.

In this analysis I assume that the fluid angular velocity within the Sun has a radial dependence of the form $\mathbf{\Omega} = \Omega(r)\hat{z}$. The axis $\hat{z}$ is known from surface motion. For a rotation law of this form, the frequency of a free-oscillation mode depends on the quantum numbers (nlm) of the mode as[7]:

$$\nu_{nlm} = \nu_{nl} + m\Delta_{nl} \tag{1}$$

where Δ_{nl}, defined in an inertial frame, is an integral over radius of $\Omega(r)$ times a weighting function. Explicit expressions for the weighting functions, or splitting kernels, are given in ref. 7. (Other sources of splitting, such as a magnetic field, will be ignored in this analysis.) For rotation curves which are suitably smooth functions of r, the low-l 5-min *p*-mode splitting is adequately approximated by the expression[8]

$$\Delta = \int_0^R \frac{\Omega(r)}{2\pi} \frac{\mathrm{d}r}{c(r)} \Big/ \int_0^R \frac{\mathrm{d}r}{c(r)} \tag{2}$$

in which $c(r)$ is the sound speed at radius r. I further assume that the modes are excited independently and isotropically, and that the mean energy per (nlm) mode depends only on frequency, in accordance with the overall distribution of power in peaks of different l as observed in the power spectrum of both the Nice data[8,9] and the ACRIM data.

The present conclusions come from measurements of the width of several $l = 0$ and 1 peaks near the maximum of the 5-min mode excitation envelope (~3,100 μHz). The $l = 2$ peaks were not quantitatively analysed because of their small signal-to-noise ratio. Because the axis $\hat{z}$ is nearly perpendicular to our line-of-sight, only even $l + m$ components should be visible in whole-disk measurements. I therefore assume the underlying line profile (in excess of background) of each $l = 1$ peak to be a superposition of two identical lobes, representing the $m = \pm 1$ components. The observable separation of the lobes, S, is related to the sidereal splitting Δ in equation (1) by

$$S = 2(\Delta - \nu_{\rm e}) \tag{3}$$

where $\nu_{\rm e} \approx 0.03$ μHz is the orbital frequency of the Earth about the Sun. The separate lobes are assumed to have lorentzian line shapes. The $l = 0$ peaks are represented by a single such lobe. Defining the linewidth as the width of a symmetrically placed bin containing half the power in the line[2], the width of the assumed doublet profile, W_1, the width of the individual lobes,

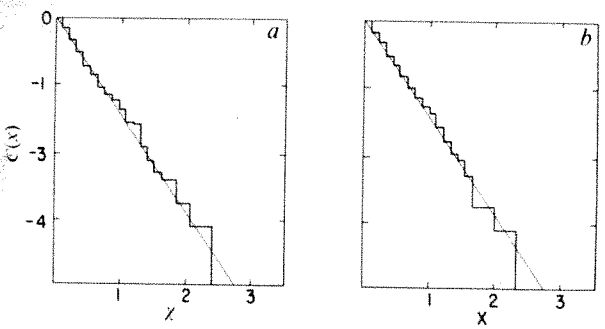


Fig. 1 *a*, The logarithm $C(x)$ of the fraction of frequency points for which the spectral power estimate p'_ν exceeds the true power by the factor x (arbitrary units), plotted for frequencies taken from 1.86-μHz bandwidth intervals centred on 5-min *p*-mode resonances in the periodogram of ACRIM data. (The true spectral power p_ν was determined from the mode parameter estimation scheme of ref. 2.) Eight intervals were selected, corresponding to the peaks $l=0$, $n=20$–23 and $l=1$, $n=19$–22. The frequency sampling of the periodogram is 29.1038 nHz so that 64 frequencies per mode were used. *b*, $C(x)$ plotted from 512 frequencies belonging to a background interval in the 5-min range. The linearity of these plots corresponds to a χ^2 distribution with 2 degrees of freedom, the distribution assumed in the error analysis. (This method of display was suggested by P. Delache.)

W_0, and the separation, S, obey a pythagorean relationship:

$$W_1^2 = W_0^2 + S^2 \quad (4)$$

The differences between the shapes of 5-min peaks in the ACRIM data and the idealized line profiles discussed above are assumed to be mainly statistical: thus linewidth measurements are subject to random error. Accordingly, I assume that the spectral power estimates p'_ν at frequencies separated by more 0.04 μHz (the resolution of 290 days of data) are uncorrelated, and that p'_ν at each frequency ν comes from a χ^2 distribution with 2 degrees of freedom, a distribution typical of a periodogram of filtered noise. The mean of the χ^2 distribution, p_ν (the true spectral power that p'_ν estimates), presumed to be a superposition of idealized 5-min mode profiles and a nearly flat background 'continuum', was estimated from the parameters of the individual 5-min modes[2] and the observed continuum.

Two tests demonstrate the credibility of these statistics. To check that the periodogram conforms to the stated distribution I have plotted $C(x)$ against x in Fig. 1, where $\exp\{C(x)\}$ is the fraction of periodogram points for which p'_ν/p_ν exceeds x. The straight line plots confirm the expected $C(x) \propto -x$ dependence, both for frequency intervals containing 5-min modes and for background intervals. To verify the statistical independence of the spectral estimate at frequencies differing by more than 0.04 μHz, I smoothed the periodogram and measured the decrease in the scatter of the points. The ratio of the variance in the smoothed periodogram to that in the original should equal 0.04 μHz divided by the effective resolution of the smoothed periodogram. This dependence was confirmed for frequency ranges of interest.

The operational definitions of power and linewidth[2] systematically underestimate the true power and width because the power in the modes is integrated only over a 5-μHz interval. A Monte Carlo simulation was performed to calibrate empirically the relation between the true power and width of the $l=0$ modes and the expected value of the operationally defined power and width, and to determine the random error of the measurement. In this, and in subsequent simulations, a statistical ensemble of artificial periodograms containing modes and background were generated with the statistical properties assumed for the real data. Ignoring the possible variation of linewidth with frequency, the true width inferred for the four strongest $l=0$ lines ($n=$ 20–23) is $\Delta\nu = 1.6 \pm 0.3$ μHz.

A second Monte Carlo calculation calibrated the relation between the width W_1 and power of the $l=1$ doublets and the operationally-defined linewidth and power, assuming that the

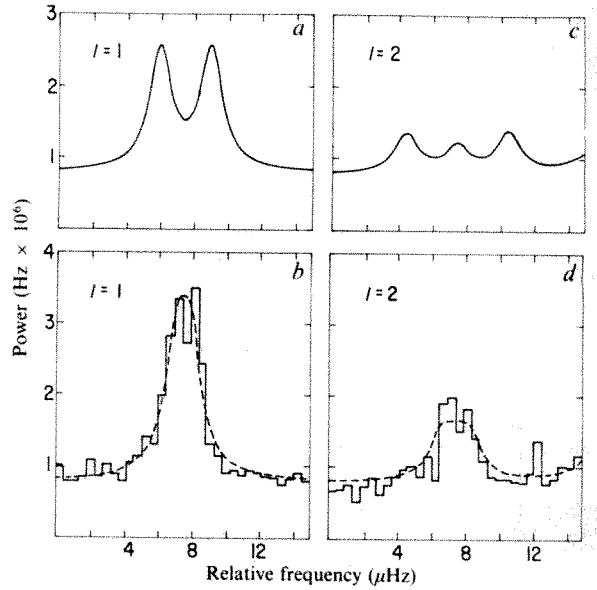


Fig. 2 *a*, Theoretical $l=1$ profile with a lobe separation of 3.0 μHz, that is a splitting $\Delta = 1.56$ μHz as defined in the text, slightly less than the separation implied by the rotation curves of refs 5 and 6. The total power in the doublet equals the mean power of the $l=1$ modes studied in this analysis and the width of the lobes equals the estimated 1.6-μHz width of the $l=0$ modes in the same frequency range. The continuum power level is that of the ACRIM data. The theoretical line shape corresponding to the larger splitting stands in marked contrast to the appearance of the actual modes. *b*, Superposition of three $l=1$ peaks ($n=20$–23) in the ACRIM periodogram, based on the measured frequency centroids, plotted at a resolution of 0.4657 μHz. The smooth curve is a doublet with a (synodic) lobe separation $S = 0.86$ μHz, the separation expected from solid-body rotation with a ~25-day sidereal period and represents the theoretical appearance of a smoothed periodogram based on an unlimited amount of data. *c*, Idealized $l=2$ profile resulting from the $\Delta = 1.56$-μHz splitting is also at variance with the data and further substantiates our conclusions. The relative power 3:2:3 in the $m=-2$, 0, and +2 lobes shown and the absence of the $m=\pm1$ lobes in full-solar-disk measurements is dictated by isotropy and by the assumption of a solar rotation axis perpendicular to our line of sight. *d*, The superposition of the four $l=2$ modes ($n=19$–22) is also consistent solid-body rotation (the smooth curve again indicates the theoretical profile which best fits the data).

doublet components have the same width as the $l=0$ modes in the same frequency range (that is, $W_0 = \Delta\nu$). This gives a mean width for the three $l=1$ modes ($n=20$–22) of $W_1 = 1.9 \pm 0.2$ μHz. A composite of the three observed $l=1$ modes appears in Fig. 2. The value of Δ inferred from W_0 and W_1 through equations (3) and (4) is 0.5 (+0.2, −0.5) μHz, consistent with the 0.456-μHz splitting expected from solid-body rotation with a ~25-day sidereal period, but also consistent with any rotational rate between 0 and 1.4 times the surface rate (1σ error limits). Note that only the magnitude of S (in equation (3)) can be determined from the (full-disk) ACRIM data. An unambiguous value for Δ was obtained by assuming that S is positive, in accordance with a net internal rotation having the same sense as the surface rotation.

Relaxing the assumption $W_0 = \Delta\nu$, made above, leads to a more rigorous upper bound on splitting. The probability of obtaining an $l=1$ linewidth smaller than the observed one, for various hypothetical values of Δ, was computed from a Monte Carlo program. In this final program, the influence of oscillations aliased into the 2,900–3,300-μHz range by the ACRIM shutter operation[2] was also simulated. Based on the published analysis of the Nice group[10], whose velocity oscillation spectrum extends well beyond the 3,814.7-μHz ACRIM Nyquist limit, the spectral power per multiplet of given l is at least three times greater at the frequencies of interest than at the frequencies of multiplets that would produce aliases. The m-state width of the potentially aliased multiplets is assumed to be 5 μHz. For each point on a

grid of mode parameters (Δ, W_0, and parameters defining the power and frequency of the aliases) a number of trial measurements were performed. A trial consists of generating three independent periodograms based on the same profile, measuring the doublet width in each, and averaging the three measurements. For $\Delta = 1.0$ and 2.0 μHz, 256 and 1,024 trials were made, respectively, for each value of the other parameters. (The ranges of the other parameters reflect their uncertainty, in particular W_0 was assumed to lie in the range 0.6–1.8 μHz.) At the lower splitting only one trial (at most) per grid point resulted in a mean width smaller than the observed one, and at the larger splitting there were no such occurrences. I thus infer a strong ($\approx 2.5\sigma$) upper limit $\Delta_{max} = 1.0$ μHz on the rotational splitting.

These results demand comparison with internal rotation curves obtained from different analyses of rotationally split modes in the solar limb-darkening data[4]. Hill *et al.*[5] have derived a rotation curve from these data which implies a solar mass quadrupole moment $J_2 = (5.5 \pm 1.3) \times 10^{-6}$, in conflict with the einsteinian general relativistic interpretation of Mercury's perihelion motion at the 2σ level. From a preliminary analysis of the same data, Gough[7] obtained $J_2 = 3.6 \times 10^{-6}$, not large enough to conflict with general relativity. Both analyses used seven multiplets for the curve fitting, a common set with the exception of two *p*-modes. A more recent analysis by Campbell *et al.*[6] essentially confirms the Hill *et al.* rotation curve, giving $J_2 = 5.0 \times 10^{-6}$, again in conflict with relativistic predictions.

To test the conclusions derived from the limb-darkening data I have calculated, using equation (2), the splitting of the low-l 5-min modes implied by the curves used to derive the quoted J_2 values. The function $c(r)$ was taken from a recent standard solar model calculation[11]. The rotation curves in refs 7, 5, and 6 imply $\Delta = 1.46$, 1.70, and 1.69 μHz, respectively, in conflict with the upper limit $\Delta_{max} = 1.0$ μHz derived here. Thus the basis established in refs 5 and 6 for conflict between general relativity and solar oscillation data is removed by the SMM data.

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A new imaging proportional counter using a Penning gas improves energy resolution

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In recent years Siegmund *et al.*[1,2] have attempted to combine in one detector the position resolution of the multi-wire proportional counter (MWPC)[3–5] or the parallel-plate imaging proportional counter (PPIPC)[6,7] with the energy resolution of the gas-scintillation proportional counter (GSPC)[8–11], using light from avalanches in a PPIPC. By counting the light flashes from individual primary electrons, Fano factor limited energy resolution was obtained but only for energies below 2 keV. The position resolution was $\approx$350 μm over an active diameter of 25 mm. Sipilä and co-workers[12,13] used Penning gas[14,15] mixtures to improve the energy resolution to 11% at 6 keV. However, this was achieved at gas gains of <50, and in a single wire proportional counter (PC) without imaging capability. We have achieved similar resolution with gains up to 550 by using a Penning gas in a uniform field. Although satisfactory for energy measurement down to 0.1 keV this gain is not high enough for good position resolution in a PPIPC. We therefore use another stage of uniform field avalanching to boost the overall gain to values >10^5. The concept of two-stage avalanching was first reported by Charpak *et al.*[16] and used by Breskin *et al.*[17]. We have built and tested a laboratory version of such a detector (Fig. 1), which we call the Penning gas imager (PGI). A Penning gas and a two-stage parallel grid avalanche geometry are combined with a wedge and strip anode (WSA) position read-out system[18]. Preliminary experimental energy resolution is 12% FWHM at 6 keV (24% FWHM at 1.5 keV) and the position resolution is 100 μm FWHM at 1.5 keV. This compares with $\approx$10% and $\approx$1 mm for the GSPC and $\approx$18% and a few hundred micrometres for the MWPC. The particle background can be reduced by an estimated 99% using an anticoincidence technique.

The energy resolution of a PC is limited by both Fano and avalanche statistics. The FWHM resolution is given by

$$R_E(\%) = 236[w(F + f + g)/E]^{0.5}$$

where w is the mean energy to produce one ion pair, F the Fano factor, f the avalanche factor, g a broadening factor depending on the small scale grid or wire geometry, and E is the X-ray energy.

The value of F for argon (and conventional PC gas mixtures such as Ar/CH_4) is 0.17 and f is typically 0.65 (ref. 19). With $w = 26.2$ eV in argon this gives $R_E = 35E^{-1/2}$ where E is in keV. This assumes $g = 0$. In practice this resolution is not achieved due to irregularities in the anode wires[20] or the grids[7]; 18% at 6 keV is a typical value and this implies a g factor equivalent to 10% extra broadening.

In the GSPC the f and g factors are eliminated by not using avalanches. In principle, $R_E = 16E^{-1/2}$ can be achieved but a typical observed value at 6 keV is 10% compared with a theoretically possible 6%. This corresponds to an additional broadening of about 8% due to gas impurities and geometry dependent light losses in the counter.

The energy resolution of the PGI is potentially as good as that of the GSPC. By using argon with the Penning admixture C_2H_2 which has an ionization potential lower than the energy of the photons emitted by the excited Ar atoms, the admixture can be photo-ionized. Also metastable argon atoms can collisionally ionize the ethyne molecules. This process, known as the Penning effect, produces a dramatic increase in α, the first Townsend coefficient[15]. As an example, the changes in the relative efficiencies of the various processes such as excitation and ionization in a Ne/Ar mixture are shown in Fig. 2. In the initial ionization, more of the available energy of the primary photo-electron and Auger electron is used for ionization (the value of w is reduced from 26.2 to 20.3 eV)[19] and in addition the fluctuations in the energy losses are smaller. The Fano factor is thus reduced[19].

In the avalanche, using the model of Alkhazov[19] and the data on α of Kruithof and Penning, Sipilä[13] shows that for an optimum value of the field strength, f approaches zero in a (Penning) mixture of Ne and Ar. As the process for Ar/C_2H_2 is similar such a result can also be expected for this mixture. A theoretical energy resolution of $10E^{-1/2}$ is then available. Sipilä's results were obtained at low gas gains (<50) but the resolution in conventional PCs rapidly degrades with increasing gain. This is due to the strongly varying field near the anode wire which makes it impossible to use the optimum field[15,19] needed for low values of f. Sipilä was aware of this limitation and suggested the use of a uniform field avalanche[13]. In the PGI the parallel geometry provides a uniform field and much higher gains can

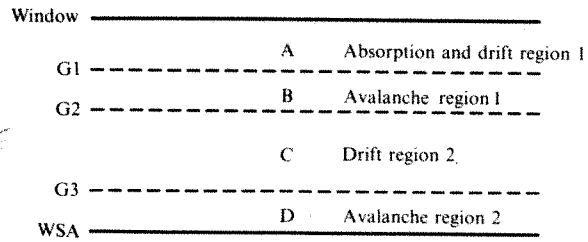


Fig. 1 Schematic diagram of the PGI. X rays enter through the thin polypropylene window and are absorbed in region A. Electrons from the resulting ionization cloud drift in a weak field through grid G1 and avalanche in the higher field of region B. The energy signal is taken from G1 and/or G2. About 20% of the avalanche electrons are effectively transferred to the second drift region C where the cloud is allowed to diffuse before reaching the second avalanche region D. The position of the electron cloud's centroid is determined using the signals from the WSA.

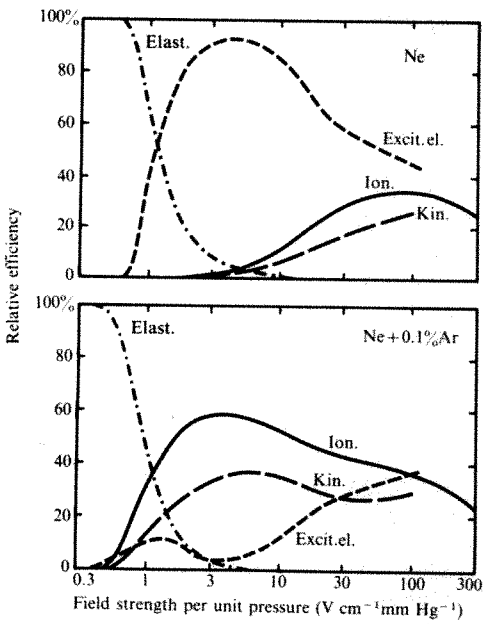


Fig. 2 Fraction of the total energy received by the electrons from the electric field which is spent in elastic collisions (Elast.), excitation of electronic levels (Excit. el.), excitation of vibrational levels (Excit. vib.), ionization (Ion.) and increase of kinetic energy of the electrons (Kin.). (From ref. 26.)

be used without the accompanying increase in R_E. With a gain of 550 we have obtained 12% FWHM at 6 keV and 24% at 1.5 keV in the laboratory detector. These measurements were made using argon with 0.5% C_2H_2. The energy resolution as a function of X-ray energy is plotted in Fig. 3. With a g factor providing a 10% broadening (we have measured 18% at 6 keV in the PGI using Ar/10% CH_4) we expect that optimizing the grid geometry would improve the energy resolution to <10% FWHM.

The gain of the first avalanche stage is set at ≈500, and ≈20% of the electrons produced here are transferred through grids G2 and G3 to the second avalanche region D. Due to the Penning effect, UV photons which pass through G2 ionize admixture molecules in region C. This process is independent of any applied field. In contrast, electron transfer in conventional counter gases can at best only take place in the ratio of the field strengths below and above the grid[21]. It has been shown[22] that with their multi-step chamber Breskin *et al.* required a Penning gas to obtain transfer at all. This is because their grids were composed of thin wires onto which the avalanches took place (not in a true parallel field) and this has the effect of reducing the transfer in a Penning gas to that of a conventional gas.

The second stage of amplification is used to boost the overall gain to $>10^5$. This is necessary for the WSA position read-out

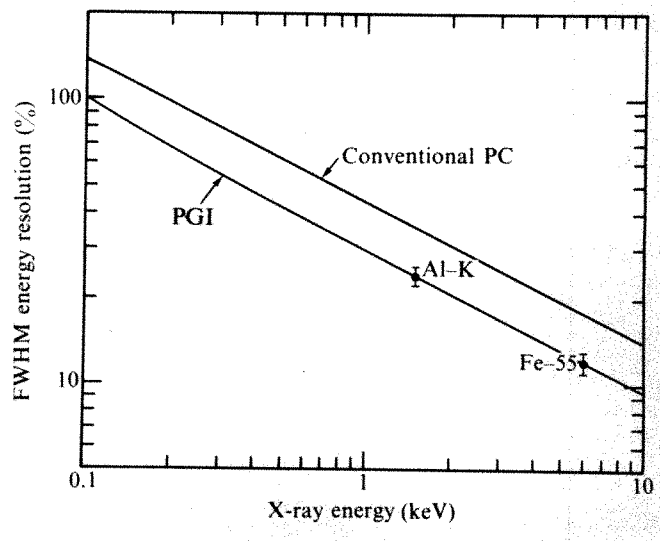


Fig. 3 The energy resolution of the PGI as a function of X-ray energy. Indicated are the resolution curves for a conventional proportional counter (PC) and for the PGI. The slight upturn in the PGI curve is due to the electronic noise contribution.

system which consists of three conductive electrodes deposited onto an insulating substrate. Their geometry is such that a linear, two-dimensional positional variation in charge output is obtained, allowing the position of an event to be determined by a simple algorithm. Preamplifier noise is associated with this charge measurement. In addition the division of charge among the discrete electrodes is binomially distributed and this gives rise to a contribution to the position resolution called partition noise.

In the same laboratory detector (70 mm diameter) we obtain a position resolution of 100 μm FWHM at 1.5 keV with a gain of 1.3×10^5. We have also built a PGI with a 200 mm diameter WSA whose overall noise is such that for all but the lowest energies (<0.5 keV) the resolution is dominated by electron diffusion in the absorption region (A) while at the higher energies the effect of primary electron track length[23] is dominant. This represents >1,000 FWHM resolution elements over most of the energy range.

In space, the background count rate due to charged particles is a problem for X-ray astronomy. This background is most effectively reduced[24,25] by surrounding the X-ray detection volume except for the entrance window by anticoincidence guard detectors. In the case of the PGI we are able to surround the WSA by an annular anode and use it with the second drift/avalanche region to provide such a detection volume. By comparison with previously flown detectors using this type of background rejection scheme, we expect to be able to reject >99% of the particle events in space[24,25].

Finally, several further improvements to the PGI are under consideration. First, the use of a xenon based Penning mixture as the filling gas will increase the quantum efficiency (QE) of the detector at high energies (in argon the QE is typically 70% at 1.5 keV and 35% at 6 keV). Second, a fast high-voltage switching circuit can be used to make the number of electrons reaching the WSA independent of the X-ray energy. This feedback system reduces the dynamic range needed for position signal processing by a factor of about 80. Third, cooling of the first stage preamplifier would improve the energy resolution at low X-ray energies by reducing the contribution due to electronic noise.

We are designing a flight version of the PGI which, with an imaging X-ray telescope will form the payload of an Aries sounding rocket to be launched in 1985.

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Effect of a heated patch of auroral ionosphere on VLF-radio wave propagation

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In the early 1960s, during the period of atmospheric nuclear tests, much theoretical interest developed in the effects of localized ionospheric depressions on the propagation of very low frequency (VLF) radio waves[1–4]. Similar VLF-propagation effects are also produced by the localized dumping of electrons from the radiation belts after wave–particle interactions[5,6]. Both nuclear explosions and particle precipitation events are of a transient nature, however, and no experimental study has yet been made to confirm these early theoretical predictions. With the development of a unique high frequency (HF) heating facility near Tromsø, Norway, the generation of movable controlled anomalies in the *D*-region has become possible. We describe here some initial observations, made in Norway, of the effect of such a movable *D*-region anomaly on the VLF signals received from the 12.1-kHz Omega transmitter at Aldra. The observations confirm the validity of earlier theoretical predictions.

Figure 1 shows the region of northern Scandinavia pertinent to the experiment. The VLF receiver used for the measurements was located at the Skibotn Astronomical Observatory, 443 km north-east of the Omega VLF transmitter at Aldra. The HF heating facility was located at Ramfjord, 15 km south of Tromsø.

The heater antenna array consisted of six rows of six crossed full-wave dipoles[7]. By suitably adjusting the relative phases of the drives to the different rows of the heating antennas, it was possible to deflect the heating beam ±56° from the vertical in the north–south plane[8]. Assuming a height of 80 km for the region of maximum *D*-region heating[9], the volume of maximum heating effect, defined by the −3 dB points, could thus be displaced 120 km north or south of Ramfjord. However, while the heated region was circular with ~20-km diameter when the beam was vertical, the heated volume had an elliptical cross-section with major axis of ~70 km and minor axis of ~38 km (see Fig. 1) when the beam was displaced 56° south or north. The experiment reported here simply entailed observing the phase and amplitude of the normal magnetic component of the

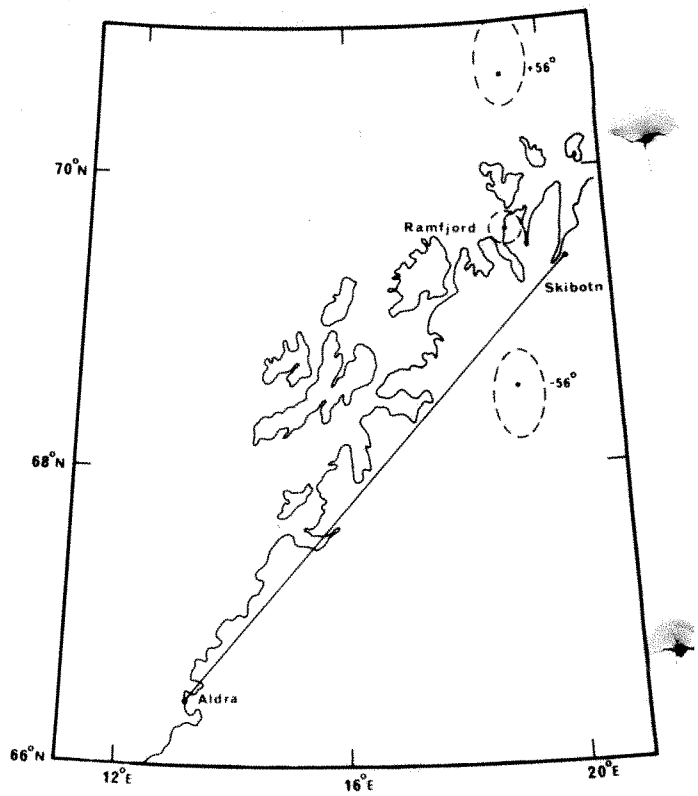


Fig. 1 Map of northern Scandinavia showing the location of the Omega station at Aldra, the VLF receiving station at Skibotn and the heating facility at Ramfjord. The dashed circle and dashed ellipses are approximate representations of the antenna −3 dB (half power) points at 80 km. The circle applies to a vertical beam, the ellipses to a beam deflection of 56° from the vertical to the north or south of the transmitter.

12.1-kHz signals from the transmitter as the heater beam was deflected across the Skibotn/Aldra propagation path. Due to the fixed locations of the heating facility and VLF transmitter, it was not possible to locate a land-based VLF receiver at a sufficiently high latitude that the heater modified the midpoint of the VLF propagation path. The optimum configuration adopted by Jones *et al.*[10] was thus not possible. It was therefore decided to locate the receiver at the most convenient experimental location, this being the Skibotn Astronomical Observatory. With this geometry it was decided that the total normal magnetic component of the VLF signal should be recorded with the eventual aim of analysing the data using a full waveguide analysis based on the work of Crombie[4] and Wait[3], the simpler analysis in terms of reflection coefficients not being feasible with such an asymmetric geometry.

Calculations[9] show that for an effective radiated power (ERP) of 300 MW at 2.759 MHz in the x-mode, the collisional frequency in the *D*-region increases by at least a factor of 5 over a height range of ~10 km. Assuming that this modification can be modelled as a 10-km elevation of the ionosphere over an area of 30 km diameter: this increased collisional frequency results in an increase of the effective VLF reflection height by 10 km, then according to the work of Field and Engel[11] a wave passing through the midpoint of such an elevated region would suffer a phase change ~ −0.4°. Experimental verification of such a small effect on VLF propagation thus entails measurement of VLF phases angles with a resolution of better than 1/10°.

Resolution is not the only significant parameter in the experiment, however, the phase stability during the experiment is also important. Experience suggests that long-term stability of VLF propagation in the auroral zone is not high[12,13] and thus the technique described below was used to offset the effect of ionospheric variability on a time scale of >2 s.

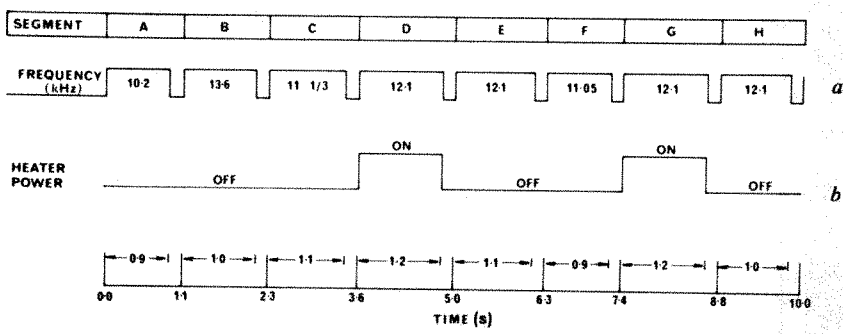


Fig. 2 *a*, Norwegian Omega transmitter format. *b*, Heating transmitter switching format. Cycles repeat every 10 s.

Figure 2*a* shows the Omega transmission format, together with the four periods of 12.1-kHz transmission. Figure 2*b* shows the period during which the heater was in operation (2.759 MHz, ERP = 200 MW). As can be seen, the heater was on for the first and third 12.1-kHz segments. By subtracting phase measurements recorded between the first and second or third and fourth 12.1-kHz segments the effect of heating could be determined over a 2-s interval, thus reducing the effect of longer-term phase variations. By operating the heater with this pulse format for 1 min, at each setting of a beam deflection angle, it was thus possible to get a mean of 12 phase difference readings at each position of the beam.

Figure 3 shows the phase and amplitude anomalies on the normal magnetic component of the Omega 12.1-kHz signal at Skibotn, recorded using the above technique. The readings were obtained from 13.45 to 14.24 UT on 29 September 1983 whilst the beam was deflected from 16 °N of Ramfjord to 56 °S. Although the maximum phase anomaly is quite small, typically ±0.5°, there is obviously a clear oscillatory variation of phase with position of the beam as predicted by the earlier theoretical works[2–4]. The maximum oscillations in amplitude are also very small, only 0.1 dB, and yet they also clearly show the same oscillatory behaviour with maximum phase displacements occurring at the same deflection angle as minimum amplitude displacements and vice versa. This 90° phase shift in amplitude and phase oscillations is in excellent agreement with the work of Wait[3] displayed in Fig. 4. The dashed and solid curves show the amplitude and phase oscillations computed for an anomaly 100 km square as it moves along the perpendicular bisector of a 10^7-m propagation path. The coincidences of maxima in phase deflection with minima in amplitude are clearly evident. (The amplitude and phase anomaly scales are normalized to those produced by a similar depression extending the full width of the path.) The experimental results can be seen to be in excellent qualitative agreement with the theory. The quantitative agreement between theory and experiment will now be considered.

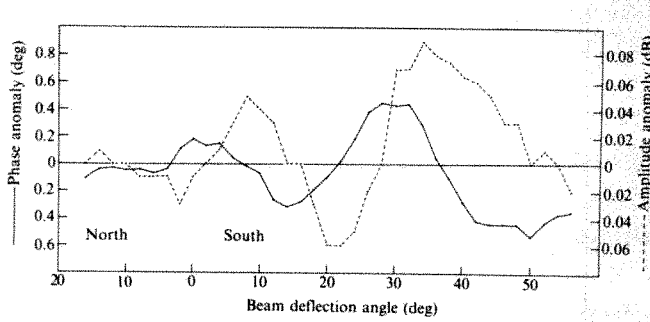


Fig. 3 Experimentally-determined values of phase and amplitude anomalies. The anomalies are plotted as a function of angular deflection of the heating beam from the vertical.

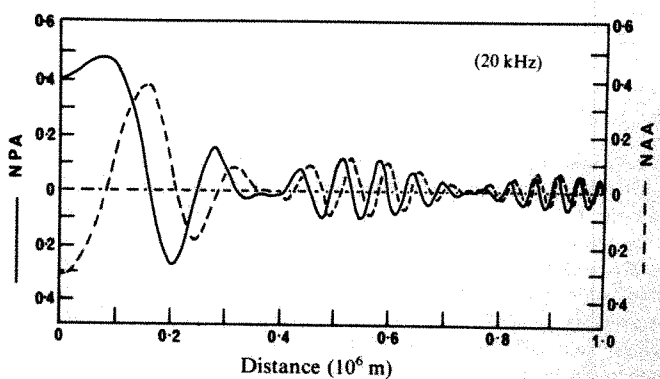


Fig. 4 Theoretical estimates of the normalized phase anomaly (NPA) and normalized amplitude anomaly (NAA) produced by an ionospheric depression 100 km square. The normalized values are plotted as a function of distance of the depression along the perpendicular bisector of the transmitter–receiver propagation path, measured from the midpoint of the path (after ref. 3).

If the effect of the heater can be modelled as a 10-km elevation of the ionosphere over a circular area of 30 km diameter, then the theory of Field and Engel[11] and Wait[1] predicts a phase anomaly of −0.4° for a ray passing through the middle of the elevated region. The heated patch in this experiment is approximately centred on the VLF propagation path for a beam deflection angle of 40 °S (see Fig. 1). At this position the patch is elliptical with major and minor axes of ~36 and ~28 km respectively. The phase perturbation of −0.42° observed at this angle (see Fig. 3) is thus in excellent agreement with theoretical predictions.

The early theoretical work of Crombie[4] and Wait[1–3] thus seems adequate to explain the phase and amplitude anomalies caused by ionospheric elevations and depressions both on and adjacent to VLF propagation paths.

The magnitude of the phase and amplitude disturbances observed here are of a similar order although somewhat smaller than those observed by Jones *et al.*[10] using the 50-MW heating transmitter at Platteville. However, they observed the phase and amplitude anomalies on the 'abnormal' magnetic component received on a 128-km propagation path when only the midpoint of the path was heated by a fixed HF beam. The total normal magnetic field was recorded in this study, which inevitably included a large component of unmodified ground-wave and this may account, to some extent, for the smaller effects observed in this experiment.

Note that the work of Wait[1–3] and Crombie[4] applies only to single-mode propagation, whereas on the short paths used in this experiment a number of modes probably contribute to the received signal even under daylight ionospheres. In nighttime conditions multimode propagation will almost certainly be significant and enhanced anomalies due to heating may be expected[6]. Similar enhancements, but invoking multipath propagation within the ionosphere, have been used to explain large amplitude anomalies on steep incidence LF data[14]. Since this initial study, further experimental observations have now been made in both daytime and nighttime conditions, and enhanced effects due to modal interference have been observed. These results will soon be submitted for publication, together with a full wave analysis of the diffraction effects produced by a heated patch of ionosphere in multimode propagation conditions.

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Delayed phase change due to hot asthenosphere causes Transantarctic uplift?

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The Transantarctic Mountains are one of the world's major mountain chains, extending 3,500 km across Antarctica in a belt 200–500 km wide (Fig. 1). The region has been uplifted by at least 4 km since late Mesozoic time, yet there is none of the thrusting, folding, regional metamorphism and andesitic volcanism associated with many other large Cenozoic mountain ranges and apparently related to subduction and subsequent continental collision. Instead, the uplift of the Transantarctic Mountains is attributed here to the delayed effects of the overriding by east Antarctica of anomalously hot asthenosphere forming under west Antarctica in the late Cretaceous. Temperature increases of 100 °C are estimated at the base of the lithosphere, with mantle heat flux increasing by ~3.5 mW m^{-2}. These changes cause an inferred uplift rate of 90 m Myr^{-1} some 50 Myr later. The late Cenozoic volcanism in the Transantarctic Mountains, typified by activity in Victoria Land, is attributed to the previously heated continental lithosphere overriding hot asthenosphere that was brought under Antarctica when it separated from Australia.

The continental uplift model used here proposes that rapid uplift will lag some 50 Myr behind the overriding of anomalously hot asthenosphere by a continent. An estimated mantle heat flux increase of 3.5 mW m^{-2} is believed to cause phase changes in the uppermost continental mantle[1]. In the absence of palaeoflow models for the asthenosphere, empirical reference frames must be used to locate hot asthenosphere and relate it to plate motions.

For interpreting the later Cenozoic uplift of southern Africa, a reference frame fixed to South America seems to be useful[1]. It has been suggested that anomalously hot asthenosphere in this frame was located at the positions of former oceanic ridges. It has also been assumed that when continental lithosphere moves over former ridge positions in this frame the hot oceanic asthenosphere is overridden by the continent rather than being carried with the oceanic lithosphere. Such a continental area would experience uplift and a higher mantle heat flux which would affect the postulated phase changes near the Moho some 50 Myr later. Later Cenozoic uplift of south-east Australia appeared to be accounted for by this model. The 'hotspots' reference frame apparently did not account for the uplifted regions of southern Africa. This frame is probably as good as the South American frame for discussing the uplift of south-east Australia, although a comparison was not made in the original work. In the case of the Antarctic, the hotspots frame of Morgan[2] gives similar results, largely because South America has not moved much relative to the hotspots frame in south polar latitudes.

Why these particular frames are useful is not well understood. They may reflect the fact that zones of upwelling asthenosphere move slowly in a fixed lithospheric angular momentum reference frame[3–5] and that South America approximates to such a frame today[6]. Their usefulness supports the assumption that hot oceanic asthenosphere can be overriden by continental plates.

Late Cretaceous to early Tertiary extensional zones have been postulated in Antarctica[7–11] to account for the development of the continental mosaic that now makes up west Antarctica. The isostatically adjusted bedrock surface of west Antarctica lies mostly at ~500 m below sea level, in marked contrast to the 1,000 m or more average elevation of east Antarctica[12]. The presence of significant volumes of volcanic rocks at depths as great as 2 km below sea level in the Byrd sub-glacial basin, suggested by seismic[13] and magnetic studies[10,11], may relate to a period of incipient ocean floor formation.

Whether or not any ocean floor was created, west Antarctica seems to have been stretched[14]. Such stretching would have brought hot asthenosphere nearer the surface. The age of the extensional zone, running along the edge of the Transantarctic Mountains is not known. It is probably no older than the separation of the Pacific plate from the Antarctic plate, whose age can be inferred to be about 91 Myr by extrapolating to closure the positions of the two plates at anomalies 29 and 32 yr (ref. 15). It is older than the anomalies now lying against the Antarctic continental edge, probably about 65-Myr old[16].

Figure 1*a*, *b* show the maximum size of the area in the hot-spots and South American reference frames previously occupied by west Antarctica that could have been overridden

Table 1 Uplift rates in the Transantarctic Mountains

Age	Locality	Observation and inference
Late Pliocene (2.5–3.4 Myr)	Wright Valley, South Victoria Land (77.5°S, 162°E)	Benthic foraminifera, originally at 10–100 m below sea level, now at 160 m (ref. 24) Uplift rate = 50–105 m Myr^{-1}
Late Miocene–early Pliocene,	Taylor Valley, South Victoria Land (77.7°S, 163°E)	Benthic foraminifera, originally 600 m now at 120 m below sea level[25]. Uplift rate in past 5 Myr of 80–100 Myr^{-1}
Miocene (15 Myr)	Miller Range, Nimrod Glacier (83.3°S, 157°E)	Glacial erosion cut at 1–1.5 km now at 2.5 km (ref. 26) Uplift rate = 80–100 m Myr^{-1}
15.7–18.3 Myr	Queen Maud Mountains (87°S, 154°E)	900 m of pre-volcanic relief; hence minimum height of region now at 2.5 km (ref. 27) Maximum uplift rate = 90 m Myr^{-1}
Post–late Cretaceous	Queen Maud Mountains (86.5°S, 150°E)	'Several thousand metres' vertical movement[28]
>25 Myr	Transantarctic Mountains	Elevation 500–1,500 m for first highland glaciers at 25 Myr (ref. 29) Uplift rate = 40–80 m Myr^{-1}

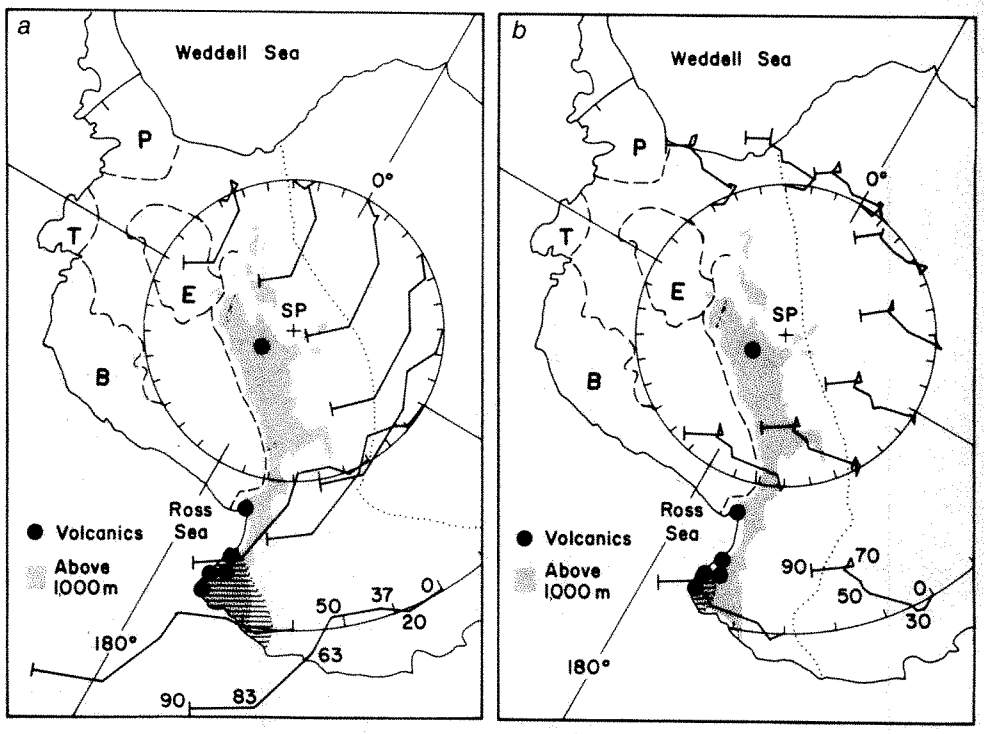


Fig. 1 The approximate outline of the Antarctic coast or coastal ice; a present-day geographical grid with latitude circles at 10° and longitudes at 10°; areas of the Transantarctic Mountains higher than 1,000 m after upward correction for the present-day ice load[12] in stipple; principal late Cenozoic volcanic fields in the Transantarctic Mountains as circles; and possible boundaries between major continental fragments as dashed lines. *a*, Solid lines show the successive past positions of points on the present-day geographical grid in a reference frame fixed to South America. Only one track has been labelled, the numbers are ages in Myr back to 90 Myr. *b*, As *a* but the tracks are taken from the hotspots frame of Morgan[2]. The dotted line in *a* and *b* shows the present position of a line that originally lay along the boundary between east Antarctica and the western edge of the Transantarctic Mountains in each reference frame. In both cases the high topography of the Transantarctic Mountains is parallel to this boundary but well within it. The horizontally shaded area in *a* and *b* is the area in each reference frame that could have overridden a former ridge position formed in early Cenozoic time.

by east Antarctica and hence subject to later uplift in the past 90 Myr. According to the model, these areas were underlain by anomalously hot asthenosphere. The uplifted area should be smaller—because of the time lag between overriding and uplift; it should form an asymmetrical mountain range decreasing in height towards the interior of east Antarctica; and significant uplift may have started within the Cenozoic, depending on the age of the extensional zone and the time-lag between overriding and uplift. The most likely age is thus about 40 Myr. As shown below, the heat needed for the phase change is small and causes negligible thermal expansion. Thus the uplift of the Transantarctic Mountains should start at a slow rate as soon as overriding begins, but will rapidly increase once the heat reaches the phase change.

Figure 1*a*, *b* show the high topography of the Transantarctic Mountains allowing for isostatic uplift following removal of the present-day ice load[12]. Several independent lines of evidence, summarized in Table 1, allow an uplift curve to be constructed for these mountains (Fig. 3).

Rates of uplift inferred from glaciological and palaeontological evidence (Table 1) are in reasonable agreement with 'erosion rates' deduced independently from fission track results on apatites in dated granite plutons from south Victoria Land[17]. They indicate slow uplift (or 'erosion rate') of 15 m Myr^{-1} for the period ~157–45 Myr. A pronounced break was detected at 45 Myr with a subsequent rate of ~93 Myr^{-1} (taking an elevation of the 100 °C isotherm at 3.8 km below sea level, based on an average geothermal gradient of 30 °C km^{-1} (refs 18–20)).

Some areas of plateau uplift are also associated with volcanism, but its timing and distribution are variable and therefore less easy to predict. In southern Africa there is no significant volcanism associated with Cenozoic uplift. By contrast, southeast Australia shows two distinct episodes of basaltic volcanism. The younger phase shows a southward migration attributed to the overriding of the hot asthenosphere originally under the Coral Sea[1]. The time-lag between overriding and the peak of the volcanism here is ~10 Myr.

The model suggests that if volcanism takes place it will be predominantly basaltic, although volcanism is not an *a priori* requirement of the model. The most probable position for volcanism is within the Transantarctic Mountains, with an age of perhaps 45–70 Myr. Later volcanism might also occur in north Victoria Land, where the previously heated lithosphere of east Antarctica has overridden hot asthenosphere brought to the base of the lithosphere by Cenozoic ocean-floor spreading at the edge of the Antarctic continent. This volcanism would have started about 20 Myr ago and should be increasing in intensity, reaching its maximum development today. The predicted extent of the area affected is shown on Fig. 1*a*, *b*.

Basaltic volcanism in Antarctica is concentrated in several provinces, although no attempt is made here to investigate it outside the Transantarctic Mountains. Here basaltic volcanism is sited principally in Victoria Land, with a minor centre in the Queen Maud Mountains (Fig. 1*a*, *b*)[21].

There is reasonable agreement between the predicted and observed timing, form and distribution of the uplift of the Transantarctic Mountains. The uplift of the Transantarctic Mountains may well have been an important factor in establishing a continental ice sheet on the Antarctic. Because stages in

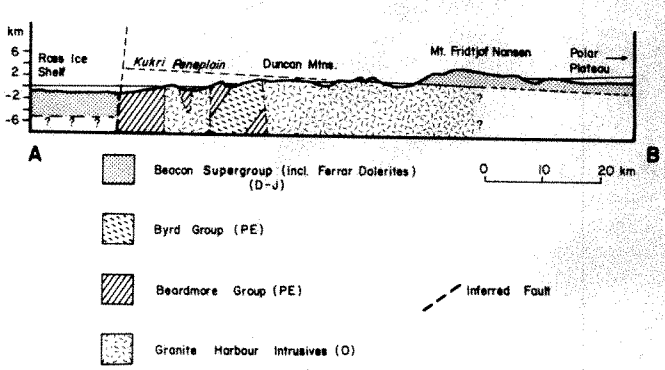


Fig. 2 Schematic cross-section through the Transantarctic Mountains at Mt Nansen (85°S, 165°W). The Kukri Peneplain separates the Beacon Supergroup (including the Ferrar dolerites) from the deformed or intrusive basement rocks. The Beacon section dips gently inland at a few degrees, from ref. 30.

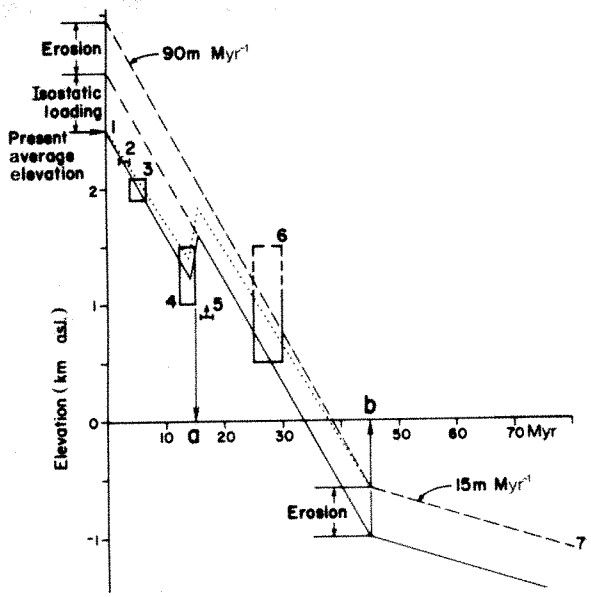


Fig. 3 Crude uplift curve for the Transantarctic Mountains. The curve has been constructed from data sources which are given in Table 1. The curve starts at the present-day average elevation of the mountains, determined as 2.5 km from a detailed analysis of USGS topographic maps (scale 1:0.25 M). The effects of the development of the east Antarctic ice sheet at about 15 Myr, assuming ice build-up over a period of 200 kyr, would have been to load the crust and depress it by an average amount of 0.5 km^2. This generates a discontinuity in the uplift curve. Before 15 Myr and back to 45 Myr the estimates, particularly from fission track studies[18], indicate a mean uplift rate also of the order of 90 m Myr^{-1}. The fission track results show a pronounced break at 45 Myr preceded by rate of 15 m Myr^{-1}. Superimposed on the uplift curve are the unknown effects of erosion. Lacking any quantitative determinations of erosion rate in the Transantarctic Mountains a global average value of 10 m Myr^{-1} has been used, but this is probably a minimum for mountainous terrain. This yields almost 500 m of surface lowering since the beginning of rapid uplift. The arrowed points locate, on the time axis, the approximate inception of a full continental ice sheet in Antarctica (a) and the commencement of the period of rapid uplift (b). Data sources: 1, present average elevation of the mountains; 2, from ref. 24; 3, from ref. 25; 4, from ref. 26; 5, from ref. 27; 6, from ref. 29; 7, from ref. 18.

ice-sheet growth can be dated and related to topography, Antarctica at present gives much more detailed information about its uplift curve than do southern Africa or south-east Australia. Unfortunately, the reverse is true of the history of the inferred extensional zone in Antarctica. Nevertheless, the changes in heat flow and temperature required for the model can be estimated.

A steady-state uplift rate of 90 m Myr^{-1} (Fig. 3) and a postulated enthalpy for the phase change of 46 kJ kg^{-1} implies a steady-state heat flux of about 3.5 mW m^{-2} (ref. 1). This is about 6% of the average heat flux. Adopting reasonable values for the thermal properties of the lithosphere, a heat flow increment of this magnitude would cause thermal expansion at a rate of ~m Myr^{-1}, which is negligible. Because most of it must go into driving the phase change, the increased heat flux will be virtually impossible to detect from surface measurements. For either the 'basalt-eclogite' or 'granulite-quartz eclogite' phase changes, 4 km of uplift implies that ~30 km of eclogite or quartz eclogite have inverted to the low density form. If the lower continental crust is of intermediate composition, then the granulite–quartz eclogite transformation is more likely[22]. The inferred 3.5-mW m^2 heat flux represents some 10–17% of the inferred mantle heat flux in continental regions[23]. With a mantle conductivity of 3.4 W m^{-1} $°C^{-1}$ (ref. 23), this would cause a temperature increase of about 1 °C km^{-1}, giving a temperature increase of about 110 °C at the base of the lithosphere.

There appears to be no early Cenozoic volcanism associated with the initial overriding of east Antarctica onto the postulated linear heat source. The nature, timing and approximate position of the late Cenozoic volcanism is reasonably well explained by the model.

Uplifted areas associated with basaltic volcanism like those of south-east Australia and east Antarctica might also enable different reference frames for inferring gross flow patterns in the asthenosphere to be distinguished. In the case of the uplift and volcanism of Antarctica, there is no clear distinction between a frame fixed to South America or the hotspots frame.

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Possible gyre–gyre interaction in the Pacific Ocean

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The subtropical anticyclonic gyres of the North and South Pacific Ocean are among the largest circulation systems found in the ocean. The gyres contain the warm waters of the warm water sphere[1,2], and the rotation of these water masses is subject to low-frequency variations[3]. The presence in these gyres of baroclinic Rossby waves with periods of many years has been documented[4,5] and this seems to be sufficient proof that the gyres undergo vacillations. Using sea-level data we demonstrate here that the subtropical gyres of the North and South Pacific oscillate with a period of about 4 years and that these oscillations are probably induced by the major El Niño events.

Oscillations of the subtropical gyre in the North Pacific Ocean have been established[3], but in the Southern Hemisphere there are insufficient hydrographic data to show that the thermal or density structure of the gyre, or its water volume, undergoes systematic low-frequency variations. There is some evidence in sea-level data of an annual oscillation between the two hemispheres, but this seems to be largely a thermal signal restricted to the surface layer[6]. Sea level along the coasts of South Australia and California shows low-frequency correspondence, which has been linked with heat and water exchange between the Pacific and the Indian Oceans through the Indonesian Waters[7].

During 1974–75 a network of sea-level gauges was established on islands in the Pacific to monitor sea-level variations[8]. Monthly mean values of sea level during the period 1975–81 were analysed by using empirical orthogonal functions[9]. The first of these functions, accounting for 42% of the non-seasonal variance, reflects an east–west oscillation of the equatorial Pacific, and its time-dependence is related to the 1976 El Niño event. Its spatial distribution closely resembles the model calculations of McCreary[10]. The second function, accounting for 11% of the variance, constitutes a north–south oscillation across the Equator. It has maximum opposite amplitudes around the Hawaiian Islands in the North Pacific and in the vicinity of Samoa and the Solomon Islands in the South Pacific. The oscillation goes through slightly more than one full cycle during the 7-yr period covered by the data, and its identification led to analysis of longer time series.

To investigate the oscillation further, the longer sea-level records at Honolulu (21° N 158° W, 1905–82) and Pago Pago (14° S 171° W, 1949–82) were subjected to a spectral analysis for the common period of data (Fig. 1). Spectral energy is high at low frequencies with periods between 3 and 4 yr. Highest coherence (0.74) between Honolulu and Pago Pago in the low-frequency range is found at a period of about 4 yr and is significant at the 87% confidence level. The oscillation at the two stations is almost completely out of phase—Pago Pago leads Honolulu by 160°. This analysis indicates that the two gyres fluctuate out of phase with typical periods around 4 yr, which is also evident from the original records and their difference (Fig. 2). Typical amplitudes of these fluctuations are 5–8 cm at each station.

We interpret the observed sea-level fluctuations as oscillations among the two large subtropical gyres of the Pacific. Each gyre constitutes a mass of warm water, which is deepest in the subtropics and near the western side of the ocean. This water mass rotates in an anticyclonic direction. If the rate of rotation or angular momentum of the gyre changes, the associated density structure must adjust because of the geostrophic balance. The

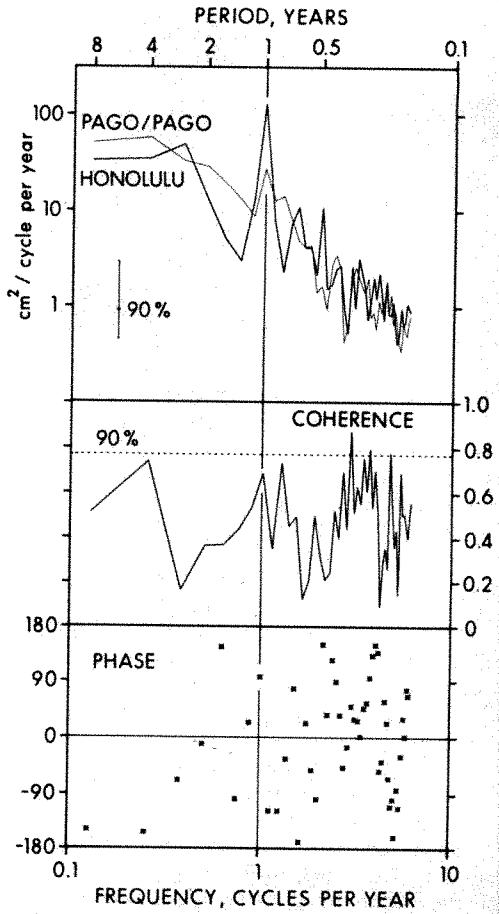


Fig. 1 Spectra of monthly sea level at Honolulu and Pago Pago for the period 1951–82, their coherence and phase for frequencies between 10 and 0.1 cycles yr^{-1}.

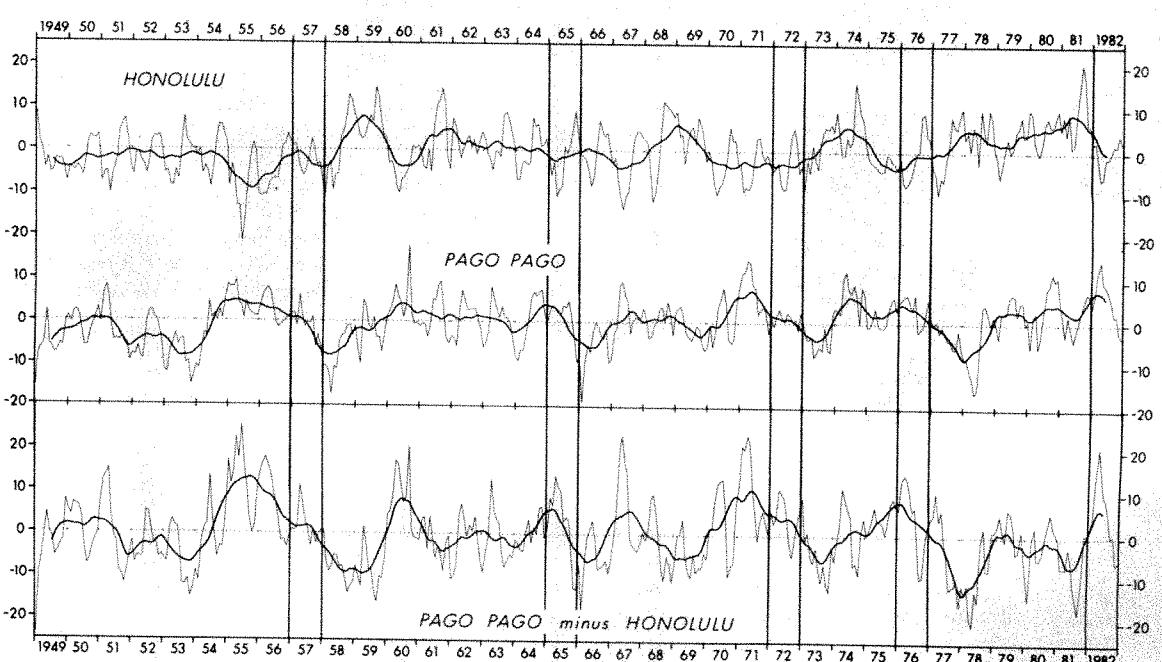


Fig. 2 Monthly mean sea level at Honolulu and Pago Pago and their difference (in cm) from 1949 to 1982. The thick curves give the 12-month running means. The major El Niño events of 1957, 1965, 1972, 1976 and 1982 are indicated by vertical lines.

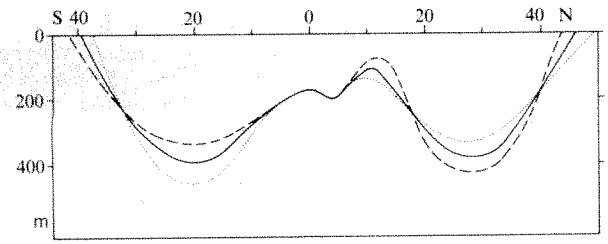


Fig. 3 The shape of the thermocline across the two subtropical gyres (solid line) and possible modes of fluctuations (dashed and dotted lines).

nature of this adjustment is sketched in Fig. 3; an acceleration of the circulation would depress the thermocline in the centre and raise it along the periphery of the gyre. A deeper thermocline implies a higher sea level because of the hydrostatic relation. If the relative density contrast between the upper layer of the gyre and the deep ocean $\Delta\rho/\rho$ is 3×10^{-3}, the depth of isopycnals in the thermocline would change 20 m for a sea-level change of 6 cm. With a depth of the gyre of 500 m, the change in the geostrophic transport of the gyre corresponding to a sea-level change of 6 cm would be $\sim4\times10^{6}\,m^3\,s^{-1}$ or about 10% of the gyre transport. Hydrographic data are inadequate to detect such small low-frequency changes in the depth of isopycnals, but transport fluctuations of similar magnitude in the North Pacific gyre have been documented[3].

Three basically different possibilities exist for the adjustment. (1) If the mass of water in each gyre remains constant, the two gyres might vacillate independently and with different periods. (2) If water is exchanged between the two gyres, this exchange has to occur across the Equator and the two gyres would be necessarily coupled. In this case the gyres have to oscillate out of phase, as is observed. (3) It is also possible that the two gyres are dynamically coupled and oscillate without exchanging mass.

It is difficult to speculate about the forcing of the oscillation. It is possible that wind torque over one of the gyres accelerates its circulation. If the two gyres were dynamically coupled, this could lead to the acceleration of the other gyre, but the oscillation would be appreciable only if a natural resonance frequency existed. This possibility could be investigated by a hydrodynamical model and the computation of the free oscillations of such a two-gyre system. On the other hand, a trans-equatorial water transport could force a coupling between the two gyres, but this coupling would not necessarily cause an acceleration or deceleration of the gyres.

The oscillations of sea level at Pago Pago (Fig. 2) follow a definite pattern related to major El Niño events. On each occasion sea level is high before El Niño, sea level drops during El Niño and is low near the end of and immediately after El Niño. In contrast, sea level at Honolulu does not show such a clear relation to El Niño events, but sea level at Honolulu peaks between such events, namely in 1959, 1968, 1974 and 1981. The difference of sea level between Pago Pago and Honolulu reflects the oscillation even better and shows its relation to major El Niño events. During each El Niño the sea-level difference is high before the event, drops during the event and is low after the event. This is the case for the major El Niño events of 1957, 1965, 1972 and 1976. Similar weaker fluctuations occur around 1960 and 1967, at a time when no El Niño occurred, indicating that the 4-yr oscillation among the two gyres is a phenomenon separate from El Niño, but might be triggered by it.

It seems possible that the north–south oscillation between the two subtropical gyres is triggered by the massive redistribution of warm water during major El Niño events. Sea-level observations of the 1982–83 El Niño show that warm water drained from the western Pacific north of the Equator during the second half of 1982 and caused a first peak of sea level in December 1982 at the Galapagos Islands. During the first half of 1983 warm water drained from the area between the Solomon Islands and Tahiti, causing a second peak in sea level at the Galapagos Islands in May 1983. During the early part of 1983 the subtropical anticyclonic gyre of the South Pacific was apparently displaced south by 10° latitude[11]. It is likely that major El Niño events trigger the oscillation among the two subtropical gyres, which continues in the years after the triggering until a subsequent El Niño event starts the cycle again. A more definite answer to this problem must await a dynamical model simulating the interaction of the two gyres.

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^{10}Be-dating of a manganese crust from Central North Pacific and implications for ocean palaeocirculation

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One of the most promising applications of the new ^{10}Be detection technique using accelerator mass spectrometry is the determination of growth rates of Mn-nodules and crusts[1–5]. Because the half life of cosmogenic ^{10}Be (1.5 Myr) is of the same order as the time needed for a few millimetres of nodule material to grow, ^{10}Be is a useful tool for unravelling such evolution during the late Tertiary, when most nodules are assumed to have started their growth[6]. Mass spectrometric measurement of a ^{10}Be profile across the manganese crust VA 13-2 from the Central Pacific, using a tandem van de Graaff accelerator, yields growth rates of 2.7 and 4.8 mm Myr^{-1} for the layers of the crust accumulated between Recent and 6 Myr BP and between 6 and 11 Myr, respectively. Using these measurements as well as ^{230}Th dating, we have been able to distinguish boundaries between zones in the crust with different structures and chemical compositions and to assign ages to them. These ages are 0.12, 2.9–3.4, 5.7–6.7, 7–9, 10–12 and 13–16 Myr BP. All of these boundaries apparently coincide with the times reported for Quaternary and late Tertiary palaeoclimatic events, suggesting that the crust growth has been strongly influenced by palaeoclimate.

During RV *Valdivia* cruise VA 13-2, 1976, a very thick ferromanganese crust (20–40 cm) was dredged near the summit of a submarine peak at a depth of 4,830 m in the Central Pacific[7,8]. In this crust, which directly overlies altered basaltic rock, three macroscopically distinguishable main zones (top zone, middle zone and bottom zone) and numerous subzones T-1 to T-6, M-1 to M-7 and I-1 to I-6 (from top to bottom) have been identified. The individual subzones, ~1–3 cm thick, are built up of macrolayers and microlayers showing a knob-like to cuspate texture similar to ferromanganese nodules. It was suggested that in the top zone (T-1 to T-6), where the ^{10}Be profile was measured, the Mn, Fe, Ni and Co and other trace elements came mainly from seawater. The Mn/Fe ratio indicates hydrogenetic growth all through the top zone[9], yet the content of Mn, Fe and of trace elements changes from one subzone to another.

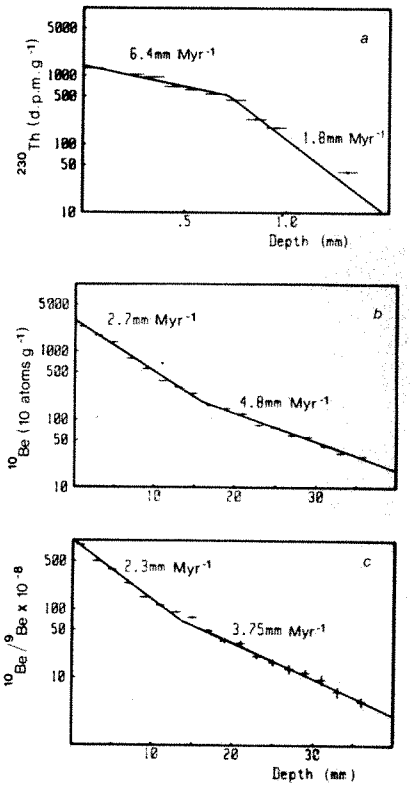


Fig. 1 *a*, ^{230}Th profile for the uppermost 1.4 mm of the crust. The exponential decrease yields growth rates of 6.4 mm Myr^{-1} for the uppermost 0.8 mm of the crust and 1.8 mm Myr^{-1} between 0.8 and 1.4 mm. *b*, ^{10}Be profile for the top zone of the crust. For the section between 0 and 16 mm the growth rate amounts to 2.7 mm Myr^{-1}, and between 16 and 38 mm to 4.8 mm Myr^{-1}. *c*, ^{10}Be/^{9}Be ratio for the top zone. It yields growth rates of 2.3 mm Myr^{-1} for the uppermost 16 mm and 3.75 mm Myr^{-1} for the depth-range 16–38 mm.

A closer look at the photomicrograph of polished sections (Fig. 3) reveals the existence of inner structure in excess of the one described by the T-1 to T-6 notation. This structure consists of a sequence of zones with 'pillar' or 'columnar' growth[8], labelled C1 to C3 and P1 to P3. We observe, for example, a clear change from pillar structure P1 to the columnar structure C2 at a depth of ~16 mm. A detritus-filled layer 16 mm from the top of the crust can be seen on the photomicro. Further layers are located at 6.5 and 22 mm (ref.8).

Samples for the ^{230}Th and ^{10}Be profiles as well as for chemical analyses of Mn, Fe, Co, ^{9}Be and Ni were taken perpendicularly to the surface using a drill bit of 1.0 cm^2 area, giving a continuous profile through the crust. Be and Th were purified using wet chromatography techniques,[2,3,10]. Th was electroplated and estimated by α-spectrometry. Be was ignited to form BeO and the ^{10}Be/^{9}Be ratios of 0.5 to 2 mg BeO samples were measured by mass spectrometry using the EN-tandem accelerator of the ETH-Zürich[11,12]. The stability of the mass spectrometer was verified by measurement of an internal standard[21]; reproducibility is better than 3%, but the absolute calibration has an uncertainty of ~10%. The sensitivity depends on the ^{10}Be contamination, which for most samples was at the p.p.m. level, leading to a background corresponding to a ^{10}Be/^{9}Be ratio of 10^{-14}. Growth rates were derived by applying half lives of 75,000 yr (^{230}Th) and 1.5 Myr (^{10}Be) and under the basic assumptions that: (1) ^{230}Th profiles and ^{10}Be/mass ratios or the ^{10}Be/^{9}Be ratio have been constant throughout time; (2) diffusion is negligible. In other words we assume that the profiles reflect only radioactive decay and crust growth.

^{230}Th yields a growth rate of 6.4 ± 0.5 mm Myr^{-1} for the uppermost 0.8 mm of the crust. Between 0.8 and 1.4 mm the growth rate amounts to 1.8 ± 0.12 mm Myr^{-1} (Fig. 1*a*). The significant change in growth rate at 0.8 mm is dated at 125,000 yr BP. The

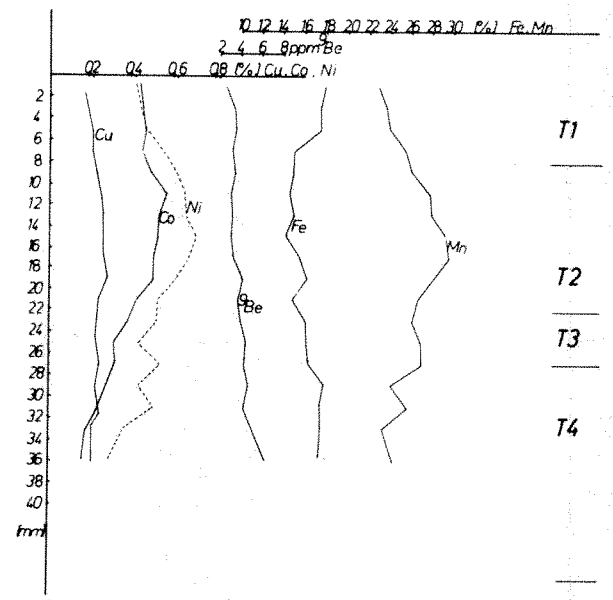


Fig. 2 Chemical composition of the top zone. The Mn content as well as the Ni content has a maximum at a depth of ≅16 mm. ^{9}Be shows a very slight but constant decrease from 4 p.p.m. at 30 mm to 3 p.p.m. at the surface, explaining why growth rates derived from the ^{10}Be/^{9}Be depth-profile are 20–30% smaller than those derived from the specific ^{10}Be content.

Table 1 Ages for boundaries of crust sections with different mineralogical structure

Boundary no.	Depth (mm)	Age (Myr)
1	0.8	0.125 ± 0.01
2	1.4	0.46 ± 0.04
C1/P1	8.0	2.9–3.4
P1/C2	15.5	5.7–6.7
C2/P2	23.0	7.8–9.1
C3/P3	36.0	10.0–12.0
Bottom of T6	42.0	11.0–14.0

Growth rates were derived from the ^{230}Th profile for the upper 1.4 mm and from the ^{10}Be-profile and the ^{10}Be/^{9}Be-ratio respectively for the deeper layers. The uncertainties of the ages result from the different growth rates calculated from the ^{10}Be/mass and ^{10}Be/^{9}Be-ratios, respectively (see Fig. 2).

^{10}Be, as well as the ^{10}Be/^{9}Be, depth profiles (Fig. 1*b*, *c*) show an exponential decrease and a clear change of the growth rate at 16 mm. The growth rate for the section 0–16 mm amounts to 2.7 ± 0.13 mm Myr^{-1} and 2.3 ± 0.2 Myr^{-1}, as derived from the ^{10}Be and ^{10}Be/^{9}Be profiles. For the section between 16 and 38 mm we derive growth rates of 4.8 ± 0.2 and 3.75 ± 0.25 mm Myr^{-1}, respectively. Applying these rates we are able to calculate ages for the boundaries of the crust sections with different texture as shown on Table 1 and Fig. 3. For comparison with other crust data from the Central Pacific[1,5,13], we compute fluxes of ^{10}Be and ^{230}Th on the surface of the crust to be 1.4×10^7 atoms ^{10}Be per cm^2 per 10^3 yr and 1.2 d.p.m. per cm^2 per 10^3 yr, derived as $F = G \times C \times D$, where G is the growth rate, C the element concentration at the surface and D the bulk density[13].

The smooth exponential decrease of the ^{10}Be/mass and ^{10}Be/^{9}Be profiles for individual larger sections as found on the crust VA 13-2 implies constant growth rate and ^{10}Be deposition. The results suggest constant ^{10}Be/carrier mass ratio for the past 11 Myr, the standard deviation from an exponential is <5%. This is a similar conclusion to Ku *et al.*[5] who reported evidence for constant deposition of ^{10}Be and ^{9}Be during the past 7–9 Myr for two analysed crusts from the Pacific and Atlantic Oceans. Further support for a constant ^{10}Be to mass ratio derived from VA 13-2 is (1) the numbers fit well Turekian *et al.*'s plot of the ^{10}Be flux against nodule growth rate on ^{10}Be dated nodules,

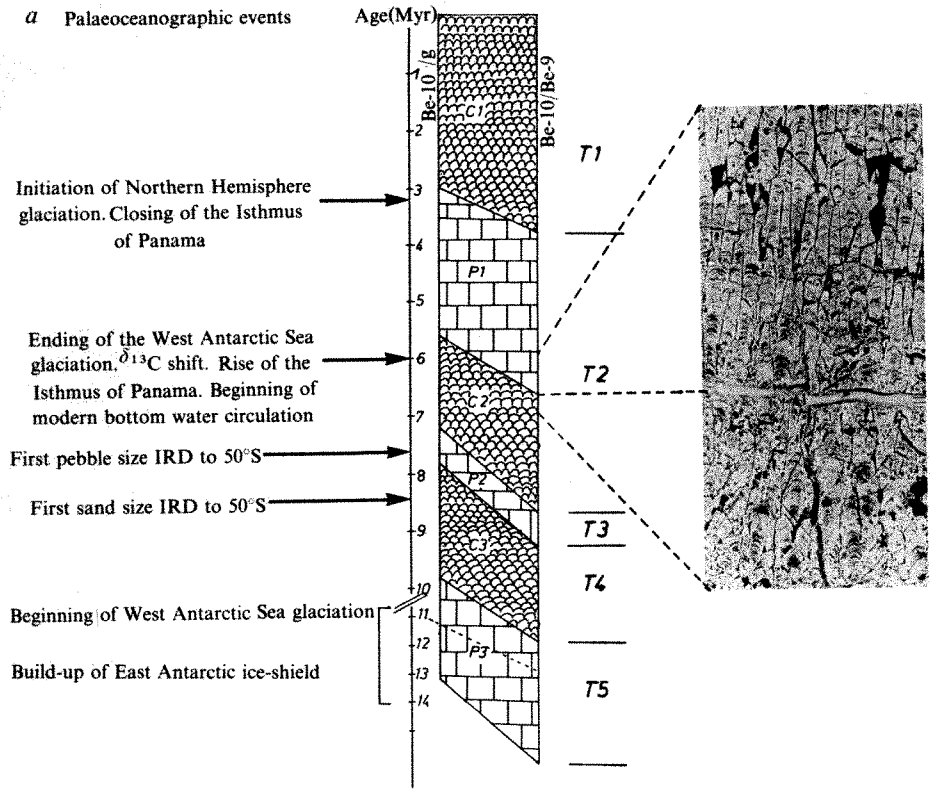


Fig. 3 *a*, Ages for the different sublayers of the top zone, calculated from the ^{10}Be-profile and from the ^{10}Be/^{9}Be ratio, respectively, and the palaeoceanographic time-markers during late Tertiary and early Quaternary. The uncertainties of the ages reflect the different growth rates derived from ^{10}Be/mass and ^{10}Be/^{9}Be ratios. *b*, Section of the photomicrograph showing the change from pillar to columnar structure P1 to C2 at 16 mm depth.

suggesting proportionality; (2) a 1.8-fold increase of the growth rate below 16 mm leads only to a different slope of the ^{10}Be depth profile and not to a significant offset of the specific ^{10}Be concentration.

One explanation would be to assume that in the water column the Mn carrier adsorbs an equilibrium amount of ^{10}Be, which is only a comparatively small fraction of the total available ^{10}Be (≅2.5%). Normalization of ^{10}Be against ^{9}Be has the advantage of making dispensable the assumption of a constant ^{10}Be to carrier ratio. It assumes, however, a constant ^{9}Be input to the open ocean and a constant concentration in seawater throughout time which, due to the comparatively short residence time of Be in the ocean, might be even more questionable than the constant ^{10}Be/mass assumption. A further crucial question is the extent to which the time record derived from the ^{10}Be depth profile may be distorted by diffusion within the crust. As has been pointed out earlier, diffusion, or diffusion associated with growth could also yield exponential depth profiles[3,4]. If we assume for the efficient diffusion coefficient of ^{10}Be an upper limit of 6.4×10^{-9} cm^2 yr^{-1} (ref. 14 and A.M., M.S., H. Kudraß, G.B., H.J.H., M.N., M.S. and W.W. in preparation), then <20% of the slope in crust VA 13-2 should be ascribed to diffusion, requiring a proportional correction of the time scale derived.

Table 2 Analytical data for Mn, Fe, Co, Ni, ^{9}Be and ^{10}Be and the ^{10}Be/^{9}Be ratio in the top zone of the crust VA 13-2

Depth (mm)	Mn (%)	Fe (%)	Co (%)	Ni (%)	Cu (%)	^{9}Be (p.p.m.)	^{10}Be (×10^7/g)	^{10}Be/^{9}Be (×10^{10})
00–02	23.02	17.95	0.43	0.41	0.16	2.7	2,320±70	142.0
02–04	23.80	17.49	0.44	0.43	0.18	3.5	1,751±30	83.0
04–06	24.16	17.49	0.45	0.46	0.20	3.6	1,384±40	63.0
06–08	25.62	15.02	0.44	0.54	0.20	3.3	791±25	39.8
08–10	26.21	15.08	0.48	0.60	0.22	3.6	560±15	25.8
10–12	27.94	14.73	0.55	0.64	0.24	3.2	370±10	19.2
12–14	28.19	15.12	0.52	0.65	0.25	3.3	300±13	15.1
14–16	29.39	14.28	0.51	0.69	0.25	3.2	244±10	12.7
16–18	29.64	15.47	0.49	0.66	0.25	3.3	162±11	8.2
18–20	28.22	16.20	0.49	0.60	0.26	4.2	146±10	5.8
20–22	26.70	14.91	0.41	0.51	0.23	3.8	122±15	5.3
22–24	26.20	16.10	0.37	0.50	0.22	4.0	83±5	3.4
24–26	26.98	16.19	0.30	0.42	0.22	4.5	77±8	2.8
26–28	27.03	16.42	0.31	0.52	0.23	4.3	59±9	2.3
28–30	24.15	17.85	0.26	0.42	0.21	4.7	56±4	2.0
30–32	25.76	17.49	0.22	0.49	0.22	4.3	41±5	1.6
32–34	23.40	17.55	0.17	0.35	0.20	5.1	32±3	1.0
34–38	24.40	17.26	0.15	0.28	0.19	6.4	29±2	0.7

Recent cross-calibrations showed that the Zürich Internal Standard yields 38% lower ^{10}Be concentrations than the standard at Rutgers University.

The data imply that the inner structure of the Mn crust may reflect global palaeoceanographic events. We shall therefore now discuss ages derived from VA 13-2 by taking as a frame of reference the comprehensive compilation of major Miocene to mid-Pleistocene events and episodes of Ciesielski *et al.*[15].

Sublayer T-1/T-2: The change from columnar zone C1 to pillar zone P1 is dated to 2.9–3.4 Myr, which corresponds with much evidence for beginning of the Northern Hemisphere glaciation[16].

Sublayer T-2: Structural change from pillar P1 to columnar C2 zone correlates with a different Mn-supply. The age of 5.7–6.7 Myr relates it to a most remarkable shift in δ^{13}C in the $CaCO_3$ in the Pacific Ocean dated to 6.2 Myr (refs 17–19). At this time the West Antarctic Sea glaciation ended giving rise to what is presumed to be the beginning of the modern bottom water circulation. Tectonic activities led to a rise of the Isthmus of Panama. This date also lies within the time period 7.4–6.2 Myr where Ciesielski *et al.*[15] assume that the main phase of erosion on the Maurice Ewing Bank occurred. Most remarkable at this boundary is the change of the growth rate as determined from ^{10}Be: this change in the growth pattern seems not to be an isolated phenomenom for VA 13-2. On the SCHW-1D crust from the Pacific dated by Ku *et al.*[5], the age of 6 Myr corresponds to layer 9.6 mm from the surface and lies within their sample 12 (9.12–10.7 mm). The flatter slope for a fit to the samples below 12 suggests that a shift in growth rate has occurred.

Evidence for a change in growth pattern at around 6 Myr BP is also recorded in several other Mn-encrustations from the North, Central and South Pacific[14].

Beyond sublayer T-2 there are three further clearly visible boundaries and changes from pillar and columnar structures that we date to 7.2–8.6, 7.8–9.1, and 10–12 Myr. Extrapolation of the ^{10}Be depth profile to 43 mm depth gives an age of 13–16 Myr BP for the basis of the top-layer. The first two markers in time could coincide with the occurrence of first pebble and

sand-size ice-rafted detritus to 50° S, on the Maurice Ewing Bank[15], which are considered to be an indicator for increased Antarctic Circumpolar Circulation (ACC). The growth of the top-zone apparently began with the build-up of the East-Antarctic ice-shield[20].

Finally, note that at sublayer T-1, at 0.8 mm from the surface (≅125,00 yr BP), where ^{230}Th indicates a remarkable variation in growth pattern, Mn begins a steady increase from ≅27% (the average for the uppermost 42 mm) to ≅35% by weight at the surface[8]. This might indicate a different and larger supply of Mn in the most recent history of the crust. Although the microprobe analysis shows that this is one of the largest variations of the Mn concentration over the last 11 Myr, we have to be aware that there might have been other short-term rate variations which we are not able to determine at the present stage of sampling resolution for ^{10}Be (≅800,000 yr).

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A relationship between dunes, fire and climate recorded in the Holocene deposits of Quebec

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During the past 5,000 yr BP the Tropics have experienced an increase in aridity and aeolian activity in both maritime and continental areas[1–4]. In contrast the onset and subsequent development of post-Hypsithermal climates in northern latitudes were primarily conducive to global cooling. It is suggested here that aeolian activity in cold environments represents a direct response to this long-term cooling trend that began after 6,000–5,000 yr BP, at the end of the climatic optimum[5–9]. This study establishes the Holocene chronology of sand dunes near the outer forest limit in northern Quebec (Fig. 1). I show here that fires initiate the aeolian activity, and that the fire–dune relationship is typical of cooling period. On this basis, a palaeoclimatic reconstruction since the climatic optimum is proposed.

The chronology of aeolian activity was based on 101 ^{14}C dates. Eighty-six organic horizons were sampled from dune palaeosols that contained discrete, non-mixed, coniferous and shrub-species charcoal, and the remaining samples were non-charred wood fragments found in dune blow-outs. The ^{14}C histogram (^{14}C yr) was constructed using the methods of Ochietti and Hillaire-Marcel[10]. Aeolian phases were established considering both the cumulative statistical weight frequency of ^{14}C dates and the distribution of individual ^{14}C dates (Fig. 2).

The histogram (Fig. 2) indicates that the aeolian activity in the study area over the past 5,000 yr BP occurred episodically. Aeolian recurrences are confined to 13 periods: 4,650, 4,200, 3,850, 3,550, 3,125, 2,875, 2,425, 2,075, 1,775, 1,475, 1,175, 575 and 175 yr BP. The time interval between aeolian activity peaks varied from 250 to 450 ^{14}C yr, except between 1,175 and 575 yr BP where it was about 600 ^{14}C yr. The histogram shows three major aeolian intervals: 3,250–2,750 yr BP, 1,650–1,050 yr BP, and 750 yr BP to present (these periods are shaded on Fig. 2). The last period was interrupted for a short time around 350 yr BP. Because of the limited number of ^{14}C dates, similar long-lasting periods cannot be confirmed before 3,300 yr BP.

Most of the dune palaeosols were Dystric Brunisols[11]. If present, the eluviated horizon was thin (Ae or Aej), less than 2 cm thick, above a more or less sesquioxide-rich Bm horizon (Fe + Al pyrophosphate). Only one palaeosol was found to present a Bf horizon typical of podsol. Selected soil profiles from the various phytogeographical zones of the study area are shown in Fig. 3. Although variable, illuviated sesquioxides found in dune palaeosols rarely exceeded a value of 0.15%. Higher values were obtained in some palaeosols developed during the 2,200–1,650 yr BP period (Fagnant Lake and Great Whale River sites, Fig. 3), suggesting favourable pedogenic conditions. In all the study sites, the original soil surface was pedogenically developed. In Burton Lake, Little Whale River and Clearwater Lake sites (Fig. 3), it was the bottom soil that presented the highest pyrophosphate values compared with the dune soils found above. The development of a soil profile within the original non-ablated surface is an indication that the deposit remained for some time in an undisturbed state allowing plant colonization and soil development to occur. The time-lag between the original deposit setting and the onset of aeolian activity varied from one site to another, depending on the rates of deglaciation and of the recession of the Tyrrell Sea[12]. Accumulation of aeolian deposits above the original surface was the result of an ecological disequilibrium in a previously stable morphosedimentary unit. Some palaeosols were frost-disturbed before their burial. Frost cracks varied from 10 to 125 cm in length within the organic and mineral horizons (such as Richmond Gulf, Fig. 3). These wedge-like cracks ranged between 5 and 10 cm in width at the top, and their vertical configuration was emphasized by iron stainings. They were probably caused by seasonal frost cracks[13] that were likely to have formed in a single season, in severe cold conditions in snow-free sites. Thawing caused local subsidence and subsequent discordant bedding. Another consequence of geliturbation was observed in some soil profiles as involuted features (Minto Lake, Fig. 3). All these frost structures were observed in the following dated palaeosols: 3,210 ± 90, 3,040 ± 215, 1,780 ± 90, 1,615 ± 100, 1,400 ± 160, 1,275 ± 90, 1,210 ± 80, 1,160 ± 80, and 1,040 ± 160 yr BP. The periods around 3,125 yr BP and between 1,650 and 1,050 yr BP were particularly conducive to frost action in soils. The northernmost sites of the study area (Clearwater Lake, Richmond Gulf, Minto Lake, Fig. 1) presented more frost-disturbed palaeosols than their southern counterparts.

The presence of many buried organic horizons in dune palaeosols suggests an alternation of stabilization stages by the plant cover and erosional stages. The number of alternations progressively decreases from the Boreal Forest Zone to the Arctic Tundra Zone. Higher frequencies (14 in Burton Lake site, Fig. 3) in forested areas (respectively the Boreal Zone and the Forest sub-Zone of the Forest Tundra, *sensu* Payette[14,15]) are an indication that aeolian activity was episodic and that dune stabilization was easier than in the Tundra Zone. North of the forest limit, dune landforms are the result of a more intense and long-lasting aeolian process[16]. In all the study sites, the buried organic horizons contained charcoal (mostly coniferous and shrub remains), suggesting a close relationship between fires and aeolian activity; fire incidence is especially conducive to erosion in rather sandy areas. Although no inference can be made about the absolute fire frequency during the Holocene, the decreasing number of buried horizons from the Boreal Forest

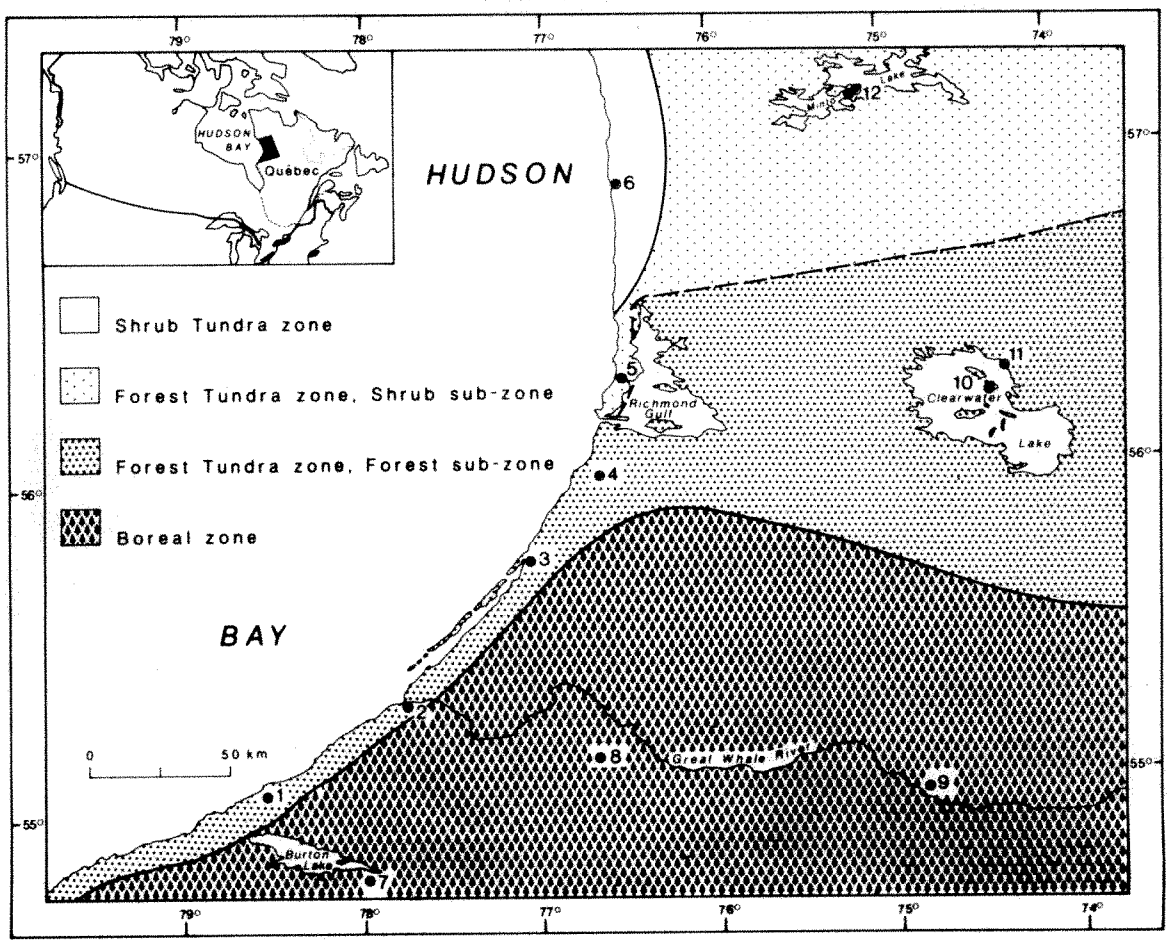


Fig. 1 Location of the study area and phytogeographical zones along the eastern coast of Hudson Bay (study sites: 1, Sucker Creek; 2, Poste-de-la-Baleine; 3, Manitounouk; 4, Little Whale River; 5, Richmond Gulf; 6, Nastapoka; 7, Burton Lake; 8, Fagnant Lake; 9, Great Whale River; 10, Ile-des-Foreurs–Clearwater Lake; 11, Nooniche–Clearwater Lake; 12, Minto Lake).

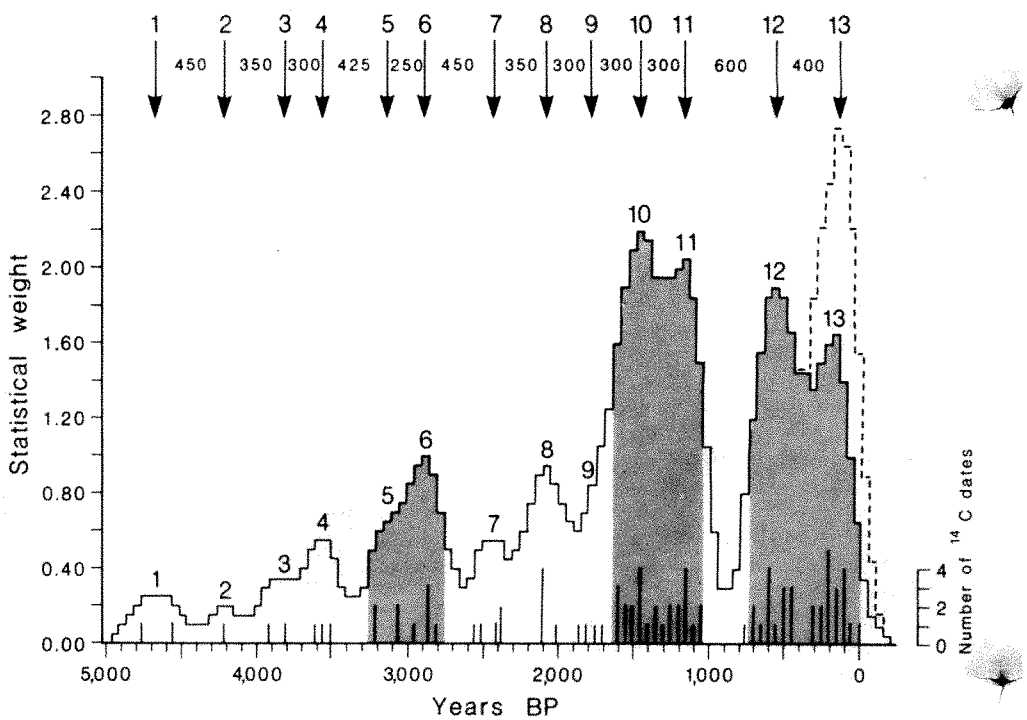


Fig. 2 Cumulative statistical weight histogram of the 101 ^{14}C dates from buried charcoal horizons (solid line) and non-charred wood fragments (dashed line). Also included the distribution of individual ^{14}C dates. Numbers from 1 to 13 refer to aeolian episodes, and shading indicates the three major aeolian episodes. The time-interval (^{14}C yr) between aeolian peaks is also presented.

to the Tundra emphasizes a decreasing gradient in fire incidence. Some long intervals of erosion-free activity, as, for example, in Little Whale River (3,610–1,160 yr BP) and Minto Lake (2,570–1,210 yr BP) sites (Fig. 3), could be the result of a lower fire frequency north of the forest limit which experienced only minor movements during the Holocene[17]. On the other hand, a detailed analysis of the regional chronologies[18] showed that greatest aeolian activity shifted from south to north between ~5,000 and

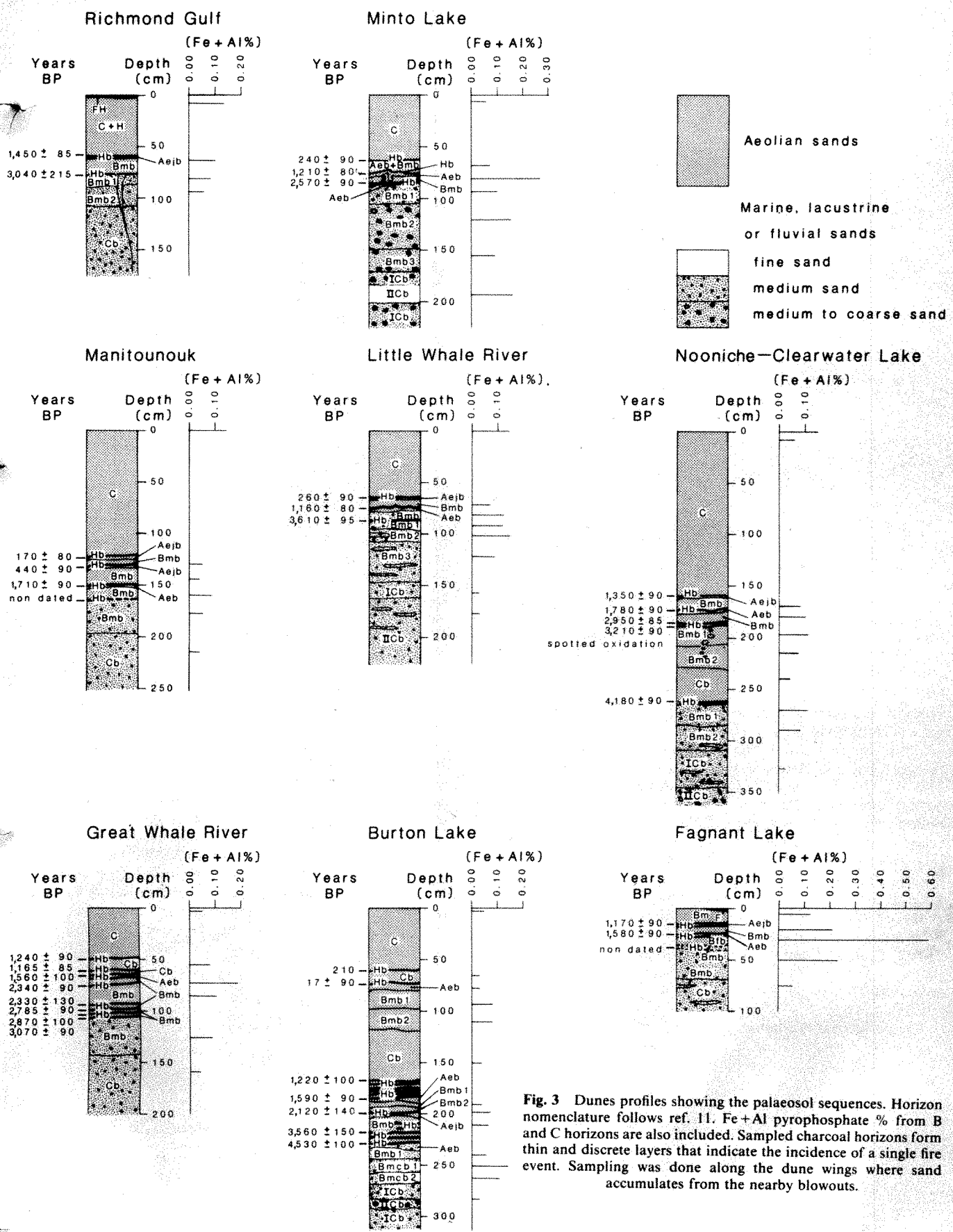


Fig. 3 Dunes profiles showing the palaeosol sequences. Horizon nomenclature follows ref. 11. Fe + Al pyrophosphate % from B and C horizons are also included. Sampled charcoal horizons form thin and discrete layers that indicate the incidence of a single fire event. Sampling was done along the dune wings where sand accumulates from the nearby blowouts.

3,500 yr BP. Aeolian activity first started around 4,650 yr BP in the Boreal Zone sites, around 4,200 yr BP in the Forest Tundra, and finally reached the Tundra Zone around 3,850 yr BP. It thus came soon after the Hypsithermal in the Boreal Zone, whereas in the Tundra Zone it began only a thousand years later. This diachronism and south-to-north displacement probably resulted from a decrease in fire incidence from the Taiga to the Tundra. The areas located in the Northern Boreal Forest and in the

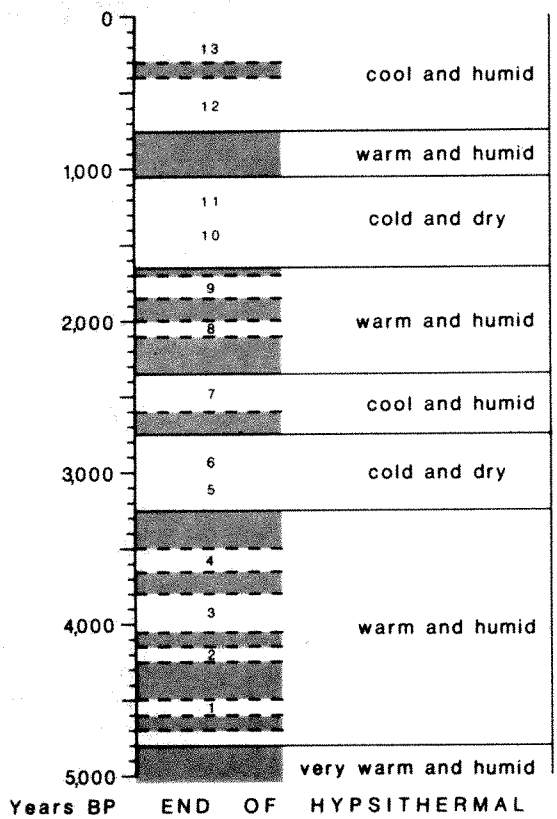
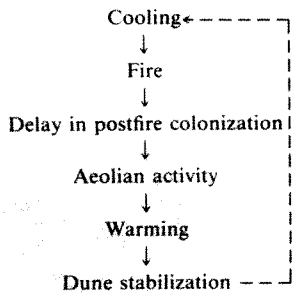


Fig. 4 Palaeoclimate reconstruction since the end of Hypsithermal. The periods delineated by a solid line are characterized by a distinct climatic regime, and the ones with a dashed line correspond to more clement (grey) and to cooling (white) intervals. Numbers 1 to 13 are the aeolian episodes.

Forest Tundra appear most appropriate for the study of aeolian activity during the Holocene, because here the forest biomass can be most conducive to the development of major fires. Here the fire frequency is relatively high, and post-fire colonization (dune stabilization) is more likely to occur during the warming periods and, inversely, may be compromised during cooling periods. Because they modify the structure of the plant landscape, fires influence the environmental equilibrium in such a way as to prevent forest regeneration. They seem to act as catalysts in inducing ecosystem change (for example, forest to dune shift) during cooling periods. This interpretation has been proposed elsewhere with regard to snow-patch expansion in northern Quebec[19], tundra-type vegetation expansion within the Forest Tundra[20], and Holocene displacements of the forest limit[17,21]. The development of aeolian systems in cold environments thus represents another ecological response to the fire-triggering process.

The periglacial aeolian cycle can be summarized as follows:



However, this cyclical model is not applicable to the tundra area. At those latitudes, aeolian systems follow a rather directional (secular) development. Climatic cooling after 3,000 yr BP in the Tundra was so important that dune stabilization was almost impossible even during brief warm periods[16,18].

Cold-region aeolian activity responds to the alternation of unfavourable climatic periods (peaks in Fig. 2) and favourable ones (lows in Fig. 2). Cold episodes follow the general, secular climatic cooling trend after the Hypsithermal (~5,000 yr BP), and became more important after 3,250 yr BP. Taking into account the fluctuations of the aeolian activity *per se*, it is possible to establish more precisely the climatic conditions that prevailed during the Holocene. Cold and dry conditions would be conducive to a significant increase of aeolian activity. The similar characteristics found in palaeosols related to the 3,250-2,750 yr BP and to the 1,650–1,050 yr BP periods are suggestive of such conditions. The presence of frost cracks during these periods would indicate severe cold conditions accentuated by a relatively dry climate (snow-free sites or with thin snow cover). Moreover, many palaeosols associated with these periods are related to a set of chronologically closely-bracketed organic horizons, emphasizing sustained erosional conditions probably after each forest fire (Burton Lake, Fig. 3, the 1,590–1,220 yr BP interval). Swain[22] also proposed that there were dry conditions in Wisconsin between 1,700 and 1,150 yr BP, based on pollen, macrofossil and charcoal evidence. A cool and humid climate would inversely decrease the aeolian activity. This trend is apparent between 2,750 and 2,350 yr BP (period 6–7, Fig. 2). The 750 yr BP to present period (episodes 12 and 13, Fig. 2) also registered a decrease compared with the 1,650–1,050 yr BP period (episodes 10 and 11, Fig. 2). This trend is more typical of maritime sites (Manitounouk, Little Whale River, Richmond Gulf, Fig. 1). More humid conditions may have accentuated the summer maritime ambiance along the Hudson Bay coast, thus reducing any fire and wind erosion probabilities (soil humidity increase). These two periods were also characterized globally by significant continental and alpine glacier expansions in mid- and high-latitude areas[6,23–25], and by snow-patch inception in northern Quebec[19]. A humid and warm climate would allow only local aeolian activity (4,800–3,250 yr BP, 2,350–1,650 yr BP) or would prevent it completely (Hypsithermal, and 1,050–750 yr BP). There is no evidence that a combined warm and dry climate ever existed at those latitudes. The palaeoclimatic reconstruction proposed here for the past 5,000 yr BP (Fig. 4), based on fluctuations of aeolian activity in northern Quebec, is presented as a first approximation. Nevertheless, it brings up the possibility of a cool/humid and cold/dry alternation in climate during the Holocene in northern, continental latitudes, as suggested by the long-term development of dune systems.

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A Gunflint type of microfossil assemblage from early Proterozoic stromatolitic cherts in China

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A well-preserved early Proterozoic microfossil assemblage dominated by a filamentous form comparable with *Gunflintia minuta* Barghoorn and a coccoid form comparable with *Huroniospora* Barghoorn has been discovered in silicified stratiform stromatolites of the Dahongyu Formation, Changcheng Group, northern Hebei Province, China. It is the oldest *in situ* microfossil assemblage in China. The famous Gunflint fossils, from the Gunflint Iron Formation, Ontario[1], were the first well preserved and relatively diverse Precambrian microbiota to be described[2,3]. Several other Precambrian microfossil assemblages are now known, but the Gunflint type has thus far been found in only a few other localities: the Duck Creek Dolomite, northwestern Australia[4]; the Frere Formation, western Australia[5]; and the Tyler Formation, northern Michigan, USA[6]. They are all early Proterozoic. The discovery indicates that the stromatolitic benthic microbiota of the Gunflint type had a cosmopolitan distribution in time between 2,000 and 1,700 Myr ago, and may have both palaeoecological and stratigraphical significance.

The sequence forms part of the northern North China Platform (Fig. 1). The fossil locality and the local geology have been described in detail elsewhere[7]. A generalized stratigraphy is given in Fig. 2. The early Proterozoic Changcheng Group consists of five formations (from the base up): (1) the Changzhougou Formation (conglomerate, sandstone and quartzite; ~170 m thick); (2) the Chuanlinggou Formation (oolitic and stromatolitic iron formation interbedded with carbonaceous shales; ~60 m thick); (3) the Tuanshanzhi Formation (dolostone, ~170 m thick); (4) the Dahongyu Formation (cherty dolostone, sandstone and lava; 110 m thick); (5) the Gaoyuzhuang Formation (cherty dolostone, ~1,000 m thick).

The Dahongyu Formation is composed of cherty dolostone, littoral to neritic sandstone, arkosic sandstone, tuffaceous sandstone–siltstone, volcanic breccia and potassium-rich trachyte. Several well preserved and distinct microfossil assemblages have been found in the lower and upper parts of this formation. The Gunflint type of microbiota occurs in the lower part of the Dahongyu Formation, in a zone 170–200 m above the top of the Chuanlinggou Iron Formation (Fig. 2). Glauconite from the middle part of the Dahongyu Formation in Jixien (eastern Hebei Province) has K–Ar ages of 1,621, 1,643 and 1,678 Myr. The U–Pb isochron ages of the upper part of the Tuanshanzhi Formation and the Chuanlinggou Formation (the underlying

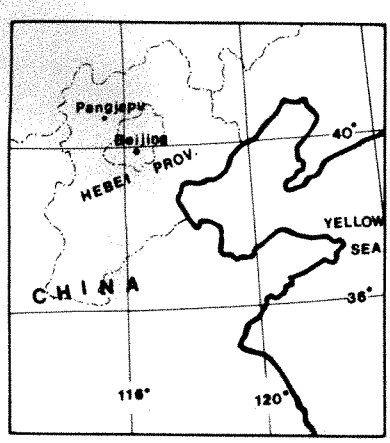


Fig. 1 Location of Pangjapu where the Gunflint type microbiota were discovered.

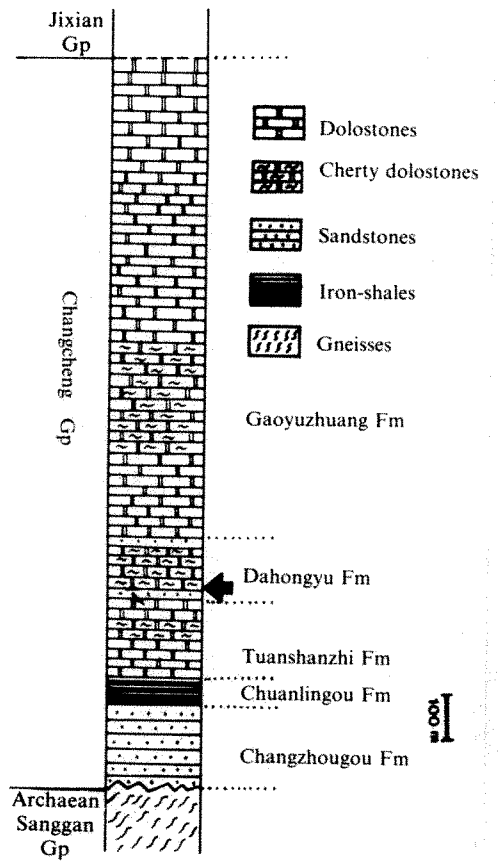


Fig. 2 Stratigraphical column of the early Proterozoic Changcheng Group in Pangjapu region. The arrow shows the position of the Dahongyu microbiota.

sediments) are 1,770 and 1,910 Myr respectively[8]. The age of the Dahongyu microbiota is therefore between 1,650 and 1,770 Myr.

The filaments of *G. minuta* and coccoids of *Huroniospora* sp. are usually found in stromatolitic organic-rich laminae. The filaments are gathered in bunches or intertwined with each other, often forming a network-like fabric (Fig. 3*a*, *b*, *f*, *k*). The coccoids are mostly scattered in spaces between the filaments. The distribution pattern of the Dahongyu microfossils is thus the same as that of the Gunflint stromatolitic microbiota from Schreiber, Ontario, suggesting that *G. minuta* and *Huroniospora* spp. were the principal mat builders.

The filaments of *G. minuta* from the Dahongyu Formation have diameters of 1–2 μm and are morphologically very similar to those from the Gunflint Iron Formation of Canada. The socalled 'heterocystous structures', that is occasional bulges in the filaments[1,9], have also been observed (Fig. 3*e*, *f*, *h*). It is possible that these structures were not original features of the *Gunflintia* organism, but instead have been caused by cell degradation. 'Budding' similar to that observed in the Gunflint Iron Formation[10] has been observed in the coccoids of the *Huroniospora* type from the Dahongyu Formation (Fig. 3*g*).

In addition to the two dominant forms mentioned above, some other rare filamentous and coccoid forms have been found (Fig. 3*i*, *j*). Some (Fig. 3*j*) can be assigned to Gunflint taxa such as *Leptoteichos golubicii* Knoll, Barghoorn et Awramik.

Two different facies of fossiliferous cherts have been recognized in the Gunflint Iron Formation: the stromatolitic chert facies containing the *G. minuta–Huroniospora* spp. dominated assemblage and the non-stromatolitic chert facies containing the *Kakabekia* and *Eoastrion* dominated assemblage[1]. The Dahongyu microbiota, dominated by *G. minuta* and *Huroniospora* spp., more closely resembles the stromatolitic benthic facies[1,2].

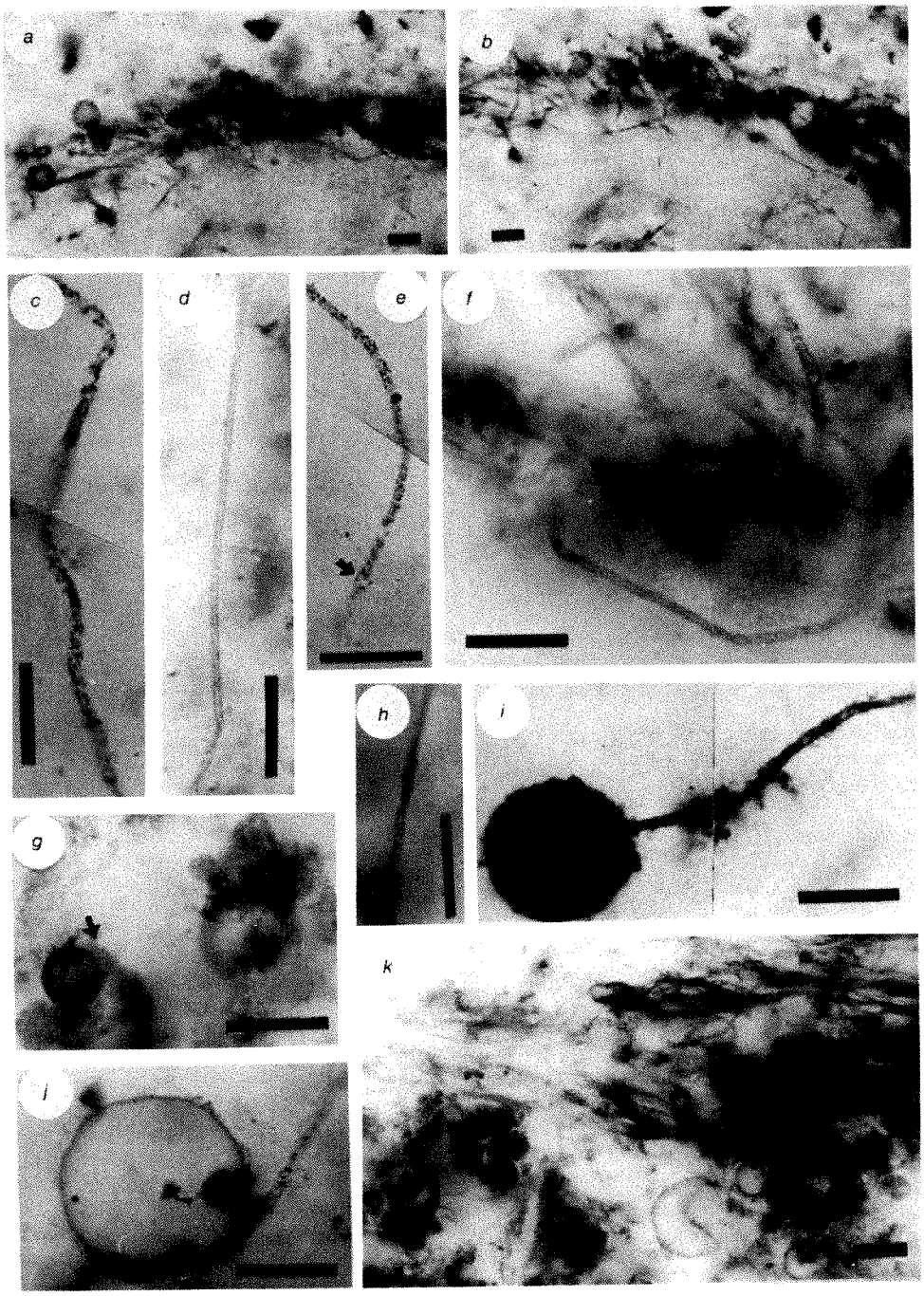


Fig. 3 Microfossils from the Dahongyu Formation. *a*, *b*, *k*, The gathered filaments of *Gunflintia minuta* and coccoids of *Huroniospora* spp, which constitute organic-rich stromatolitic laminae. *c*, *d*, *e*, *h*, *i*, *Gunflintia minuta* Barghoorn; the arrows indicate the bulges of the filaments which are similar to the 'heterocystous' structures and are probably caused by cell degradation. *g*, *j*, Some coccoid microfossils. *g*, A coccoid in budding (arrowed) similar to the budding coccoid *Huroniospora* spp. from the Gunflint Iron Formation, Ontario, Canda. *j*, A coccoid morphologically comparable with *Lepitoteichos golubicii* Knoll, Barghoorn and Awramik, a coccoid taxon from the Gunflint Iron Formation of Canada. Scale bar, 10 μm.

The discovery of a number of Precambrian microfossils over the past 20 yr has stimulated palaeontological research and raised hopes for using microfossils in studying the biostratigraphy of the Precambrian. These hopes have been tempered by the findings that some of the dominant Precambrain microorganisms show an apparent evolutionary conservatism and a considerable longevity in the fossil record. *Eoentophysalis* Hofmann[13], for example, persists for long periods and has a modern counterpart *Entophysalis*[14]. Nevertheless, other Precambrian microfossils and their assemblages appear to have been limited in time[4,15], and the Gunflint type of microbiota seems to belong to this kind. Hofmann noted the Belcher Island microbiota is significantly different from the almost contemporaneous Gunflint microbiota, but is quite similar to the microbiota from the Bitter Springs Formation, that is roughly half its age. He concluded that the Belcher and the Gunflint microbial assemblages must have lived in different environments[13].

The cherty dolostone beds in which the Dahongyu microbiota occur are intercalated with volcaniclastic sediments and lava, suggesting that the Dahongyu microbiota may have lived in a shallow marine environment in a region of volcanic activity. Evidence of contemporaneous volcanic activity is also found in the Gunflint Iron Formation and the Duck Creek Dolomite[4,16]. This suggests that the Gunflint type of microbiota may have been ecologically specialized[4,17] and may have lived in a unique environment related to volcanic activity.

The taxonomic affinity of most Gunflint microfossils has not well been defined. *Gunflintia minuta* may not necessarily be cyanobacteria, because the so-called 'heterocyst' or 'akinete' structures may be degradational artefacts, as mentioned above. The fine filaments of *G. minuta* are morphologically similar to some modern photosynthetic and thermophilic filamentous bacteria which live and build microbial mats or stromatolites at temperatures up to 70 °C in hot springs[17,18]. The budding coccoids of *Huroniospora* spp. with a wide range of diameter

(1–20 μm) may represent a taxonomically mixed population[1,6,10].

By comparing the Gunflint type microbiota from the Gunflint, Frere, Duck Creek, Tyler and Dahongyu Formations, we may conclude that: (1) *Gunflintia–Huroniospora* assemblage dominated a typically benthic mat-building microbial community that formed stratiform, columnar and spherical stromatolite morphotypes[12] in a particular range of environmental settings[4,13]. (2) The Gunflint type microbiota had a worldwide distribution in the middle Precambrian. Their fossilized remains are now found in present-day North America, Australia and China. (3) These organisms, as an assemblage association, occur within a limited time span of between about 2,000 and 1,700 Myr ago.

The discovery of the Dahongyu microbiota (the oldest *in situ* microfossil assemblage in China) provides new data for general Precambrian micropalaeontology. This supports the conclusion that these microorganisms, and possibly also the environmental conditions in which they flourished, were common and geographically widely distributed, although restricted to a relatively narrow time frame in the early Proterozoic. Thus the Gunflint type microfossil assemblage may have palaeoecological as well as biostratigraphical significance.

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The myth of the mad March hare

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From literature[1], proverb[2] and scientific publications[3–6], two aspects of the behaviour of the brown hare, *Lepus capensis*, are well known. First, they 'go mad' in March. Second, boxing is their most spectacular form of male–male competition for mates. Here we show that 'madness' is no more a feature of March than of the other months of their long breeding season, and that boxing does not represent intrasexual competition but an interaction between the sexes whereby a female attempts to prevent a male from mating. Finally, we discuss why misleading statements about hare behaviour have remained unchallenged for centuries.

The brown hare is a relatively solitary herbivore, feeding mainly at night[7]. The breeding season lasts from January to August, sometimes later, and each female may produce several litters per year[8,9]. Despite the length of the breeding season and the extensive overlap of male and female home ranges[10], it is traditionally claimed that male–male competition, in the form of chasing and boxing, peaks dramatically in March[1–6]. Indeed, scientific explanations for such activity have been offered[6].

From 1977 a population of hares has been studied on approximately 100 hectares of the flat, permanent pastures of the Somerset Levels, UK. Recordings were made from a vantage point 5 m above ground level. Data from over 1,500 hours of day and night-time observations by A.J.F.H. have been collected over 6 yr. From 1980 onwards, using an astronomical telescope giving magnifications of >100 times, and a catalogue of over 2,000 photographs of the hares, taken through the telescope, 10 males and 6 females have been recognized. Males and females are sexed by observation of male genitals and female teats. Individuals are recognized using features such as injury scars, facial markings and moult patterns.

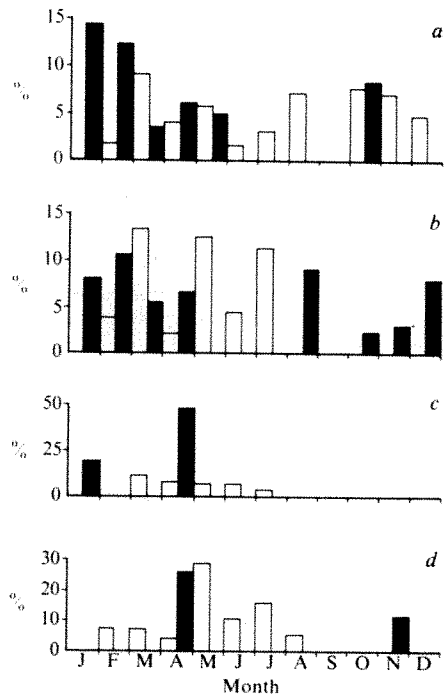


Fig. 1 The relative monthly and diel frequencies of types 1–4 (*a–d*, respectively) interactions throughout the year. Solid columns, sunset–sunrise, open columns, sunrise–sunset. Both diurnal and nocturnal samples have been standardized to take into account the amount of time spent observing each month. Cumulative hours of observation (1977–83) are as follows: January 112, February 123, March 158, April 167, May 177, June 181, July 271, August 120, September 32, October 96, November 67, December 63; total 1,567.

The different types of competitive, reproductive behaviour which occur between individuals are defined in Table 1, together with the totals of observed encounters distinguishing occasions when all individuals involved were recognized. Figure 1 shows the monthly and diel patterns of chase (types 1–3) and boxing. Of the total number of encounters, 82.1% occurred during the usual breeding period (January–August) but only 14% of the encounters were restricted to March. Of the remaining 86%, over half (52.4%) were observed at night. Nocturnal chases occurred significantly more often during winter months (October–March) than during summer months (April–September) ($\chi^2_1 = 16.31$, $P < 0.001$).

Five different females and six different males were identified for the 17 boxing bouts. In every case the boxing occurred between a female and a male; no intrasexual boxing has been observed. While we do not discount the possibility that such boxing may occur, this finding is supported by an independent, unpublished study in Oxfordshire where for each of 15 bouts in which both individuals were identified, a female–male interaction was involved (W. Reade, personal communication).

We have also studied a videotape of one prolonged boxing match lasting 1 min 59 s between a female (Camelia) and a male (Cadet) filmed by Simon King in late May 1982. During this interaction (counted as one event in the analysis) there was a sequence of 35 short, rapid chases: each chase by the male of the female alternated, as the male caught up with the female, with 34 separate boxes. In 24 cases, the female alone boxed,

Table 1 Summary of the categories of interaction between hares, the numbers observed and the occasions when all participants in an interaction were identified

		Total no. of interactions recorded	Interactions in which both or all hares were sexed and individually recognized† ♂→♂	♀→♂	♂→♀	♂♂♂→♀
Type 1 chases*:	High-speed chases between two adults which were not previously in close proximity to each other or another individual. When the hares were identified, three of four possible combinations by sex were recorded	55	8	4	2	–
Type 2 chases:	High-speed chases between two adults, one of which at the inception of the chase was in close proximity to a third adult. Chases frequently repeated. When hares were identified, one of the males was mate guarding a female and attempted to chase off the second male	69	46	0	0	–
Type 3 chases:	High-speed chases with more than two adults in a follow-my-leader arrangement. When hares were identified, several males were pursuing one female	8	–	–	–	4
Type 4, boxing:	One hare lunging frontally at another with its forepaws, usually from an upright bipedal position. A boxing bout sometimes consisted of an alternating sequence of boxing and short rapid chases	25	0	♀⇆♂ 17		–

* Type 1 chases were brief and not repeated and provided limited opportunity for individual recognition.
† Arrow indicates direction of chase.

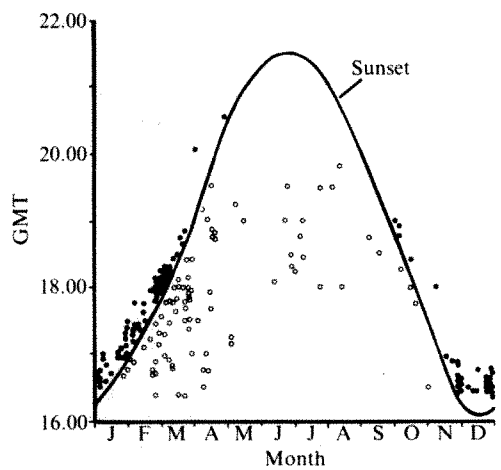


Fig. 2 Individual records (1977–83) of the times that hares were observed to leave their daytime forms in relation to the time of sunset throughout the year. This emergence from forms is equivalent to the onset of activity. ●, After sunset; ○, before sunset.

beating the head of the male with her paws as he lunged forward beneath her. In eight cases the two hares started boxing simultaneously while in the remaining two cases the male started to box after the female had initiated. In neither this case nor the other 24 boxing bouts did mating occur at the end of the encounter.

The classical view of hare 'madness' has been one of a frenzied but short period of male fighting and chasing. Indeed during the nineteenth century this episode in March was referred to as a rut[2]. On theoretical grounds such intensive behaviours should occur in polygynous species where females are available for mating for only a short, synchronous period[11]. The fact that the hare's breeding season is prolonged and involves several litters per female with limited reproductive synchrony should have suggested an anomaly in the behaviour of males, which would be expected to compete for mates throughout the breeding season—our results support that expectation. Clearly, much of the hare's behaviour has been overlooked. Early in the year this is probably because most interactions are nocturnal (Fig. 1): this viewpoint is supported by Fig. 2. The fact that hares leave their resting places (forms) in the early evening throughout the year means that activity in winter starts significantly more often after sunset than in the summer ($\chi^2_1 = 36.3$, $P < 0.001$). In the summer, behaviour patterns are probably overlooked because the length of grass restricts an observer's view from ground level. In the present study a high observation point and flat terrain minimized that problem.

Previously, boxing was regarded as a form of male–male competition for mates. In our study all such interactions were between a female and a male. We do not yet know whether the sex difference in boxing intensity in the filmed bout is reflected in the species as a whole, but it is worth noting that females are both larger and heavier than males and in the study population males frequently have injury scars to their ears.

In view of the results presented here, we offer a tentative, short appraisal of hare breeding behaviour. Early in the year males begin to chase other males and start to search for females. A male will guard a female prior to mating and will attempt to chase away other males. If there are several males close to an oestrous female, all of them, including the guarding male, may become involved in a mating chase. Boxing is the means by which a female may prevent either a particular male from mating with her or, when not in oestrus, any male from mating. This pattern of behaviour continues throughout the long breeding season. With apologies to Lewis Carroll, the March hare is a myth.

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Spider silk as rubber

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The silks produced by spiders are exceptional structural materials. Although their tensile strengths are similar to those of cellulose, collagen and chitin, their extensibilities are considerably greater[1,2]. Thus, the energy required to break spider silk (that is, its toughness) can be 10 times greater than for these other biological materials. The silks are crystalline proteins, with substantial portions of their polypeptide chains forming crystallites of stacked β-pleated sheets[3–6]. However, the extensibility of silk is attributed to non-crystalline regions, which appear amorphous on X-ray diffraction[7,8]. The mechanical properties and macromolecular conformation of these amorphous regions have been the subject of much speculation[1,2,7–10], but little direct experimentation. Here we show that these regions can behave as rubber networks and exhibit rubber-like or entropy elasticity.

At room temperature (~20 °C, 50% relative humidity) the silk produced by the major ampullate gland (dragline silk) of the spider *Araneus diadematus* is very stiff. If, however, an unrestrained segment is immersed in water it absorbs water and contracts to about 55% of its dry length[9,10]. This hydration-induced contraction is accompanied by a drastic reduction in elastic modulus from $\sim 10^{10}$ N m^{-2} to $\sim 10^{7}$ N m^{-2}, and a large increase in extensibility. These properties suggest that contracted silk is a protein rubber. X-ray diffraction, however, indicates that the crystalline regions are relatively unaffected by this contraction[9]. The interchain crystal dimensions are unchanged and analysis of diffraction intensities indicates that the crystals reorient but are not substantially melted. Thus, contracted silk appears to be a protein rubber filled with crystalline inclusions.

A rubber is composed of a large number of kinetically free, random polymer chains that are cross-linked to form a macroscopic network. The elasticity of such a network is due primarily to changes in the conformational entropy of the polymer chains, and only secondarily to changes in internal energy[11]. In contrast, stiff solids such as steel, store elastic energy as changes in internal energy associated with the distortion of chemical bonds. We have used thermoelastic measurements to determine the relative importance of internal energy and conformational entropy changes in the elasticity of contracted spider's silk. The elastic force (f) for a material deformed as a closed system at constant volume (V) and temperature (T) can be expressed in terms of changes in internal energy (E) and entropy (S), as follows[11]:

$$f = (\partial E/\partial L)_{T,V,n} + T(\partial f/\partial T)_{L,V,n} \quad (1)$$

where L is the length and n indicates constant composition. Thus, the slope of a force–temperature plot at constant length and volume and fixed composition, $(\partial f/\partial T)_{L,V,n}$, multiplied by temperature gives the desired conformational entropy component of the elastic force (f_S), and the intercept of the force–temperature plot, $(\partial E/\partial T)_{T,V,n}$, gives the internal energy component (f_E).

In practice, it is nearly impossible to maintain constant volume and fixed composition in a thermoelastic experiment because the swelling of a sample held in equilibrium with a swelling solvent can vary with temperature. A thermoelastic relationship written for the conditions of constant pressure (P) and swelling equilibrium (eq),

$$f = (\partial H/\partial L)_{T,P,\mathrm{eq}} + T(\partial f/\partial T)_{L,P,\mathrm{eq}} \quad (2)$$

yields entropy and enthalpy (H) components, which contain contributions from network chain conformational changes and

Table 1 Enthalpy and internal energy components of elastic force of spider silk

a Sample	α	f_H/f (uncorrected)	f_E/f (corrected)	Modulus (N m^{-2})
2A	1.098	−0.36 ± 0.14	0.07 ± 0.16	9.2×10^6
1A	1.204	−0.03 ± 0.06	0.10 ± 0.07	6.2×10^6
2B	1.211	0.01 ± 0.11	0.19 ± 0.12	8.5×10^6
2C	1.293	0.08 ± 0.10	0.19 ± 0.13	1.0×10^7
			Av. = 0.14 ± 0.12	

b Material	f_E/f
Elastin	0.26
Natural rubber	0.18
cis-1,4-Polybutadiene	0.13
Polydimethylsiloxane	0.19

a, The results calculated for contracted spider's silk, as described in the text: f_H/f was calculated from equation 2, as the intercept (at 0 K) of the uncorrected stress–temperature plot, divided by the stress at 30 °C (303 K). f_E/f was calculated from equation 3 using the data corrected to give stress at constant extension ratio. The error estimates were derived from standard errors calculated from the regression statistics for the stress–temperature and length–temperature data. The modulus values were calculated by dividing the stress at 30 °C at each extension by the strain (strain = $\alpha - 1$), to give an indication of the stiffness of contracted silk. *b*, Some selected values for the internal energy component of the elastic force for natural and synthetic rubbers. (Data from ref. 13.)

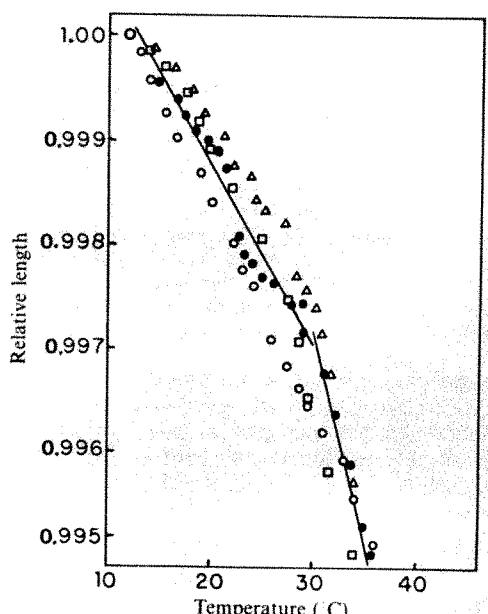


Fig. 1 Relative length versus temperature for four ~2.5-cm long samples of dragline silk immersed in distilled water. The relative length appears to decrease linearly with temperature over the range 10–30 °C, and linear regression analysis of all data up to 30 °C indicates a coefficient of linear expansion of -1.8×10^{-4} °C^{-1} ($R^2 = 0.923$, $n = 50$). Above 30 °C the magnitude of the swelling change appears to increase significantly, and regression analysis of all data above 30 °C yields an expansion coefficient of -3.9×10^{-4} °C^{-1} ($R^2 = 0.855$, $n = 15$). The cause of this change in swelling behaviour at around 30 °C is unknown, but to avoid problems we restricted our thermoelastic measurements to the region of linear behaviour between 10 and 30 °C. Swelling in this range is assumed to be isotropic, but this assumption was not tested due to the small swelling coefficient and the small diameter of the silk fibres (~5 μm).

Methods: Samples were suspended vertically in a cuvette made from microscope slides and held extended at essentially zero force by the weight of a glass rod (~ 5 mm long, 0.1 mm diameter) glued to the free end of the silk fibre. The weight of the rod caused an extension of less than 0.3%, and its effect is ignored. Changes in the length of the silk fibre were observed by following the vertical movement of its free end with a filar micrometer eyepiece on a Wild M5 dissecting microscope. The cuvette was mounted on a temperature-controlled stage and temperatures were measured (±0.5 °C) with a thermistor placed near the sample.

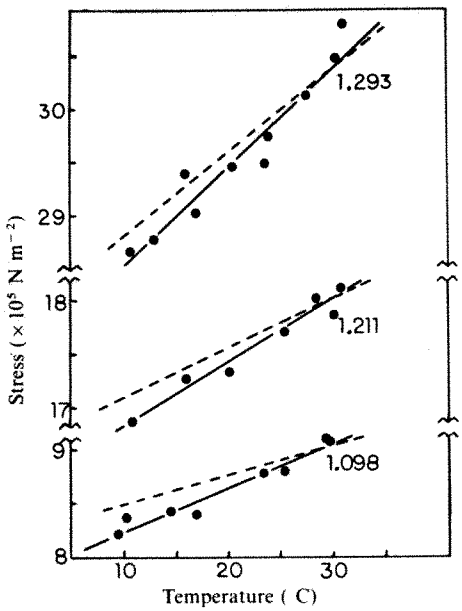


Fig. 2 Thermoelastic data for dragline silk immersed in distilled water and extended to the extension ratios indicated. Samples (~2 cm long) were mounted on a test frame, described previously[16], in which the force is determined by following the deflection of a thin (~0.1 mm diameter) glass beam. The beam deflection was measured with a filar micrometer eyepiece on a Wild M5 dissecting microscope, and sample temperature was maintained and measured as described for Fig. 1. The silk sample was extended to its test length at the highest temperature (~30 °C) and allowed to relax for about 1 h. The temperature was then lowered in stages to ~10 °C, raised in stages to ~30 °C and lowered again to ~10 °C. The forces at each temperature were calculated from the mean of 6–10 determinations of beam deflection, and in all cases the standard error was roughly the size of the point plotted. The solid lines indicate the linear regression of the data (correlation coefficients, R^2, are all greater than 0.91). The broken lines indicate the regression line corrected to give stress at constant extension ratio. The corrected data were used with equation 3 to calculate f_E/f.

also from polymer-solvent mixing processes. To determine f_E and f_S for contracted spider's silk we have used constant-pressure measurements, and then corrected for swelling changes by calculating the force–temperature coefficient at constant extension ratio (α = extended length/initial length), using the following relationship[12];

$$f_E/f = -T\left(\frac{\partial \ln (f/T)}{\partial T}\right)_{P,\alpha,eq} + T\lambda_s \qquad (3)$$

where f_E/f represents the fraction of the force due to changes in internal energy, and λ_s is the coefficient of linear thermal expansion. Because this swelling correction assumes that swelling changes are isotropic, the calculated f_E/f values are reasonable approximations but are not exact.

Figure 1 shows length–temperature data for contracted silk, from which λ_s is calculated as -1.8×10^{-4} °C^{-1} between 10 and 30 °C. Figure 2 shows the results of thermoelastic measurements on contracted silk at three extensions. The force values are divided by the initial cross-sectional area to give a value for stress. For each extension the solid line shows the linear regression of the data; the broken line is this regression corrected for the effects of swelling to give the stress at constant extension ratio, with 30 °C taken as the reference.

Table 1 gives the enthalpy (f_H/f; equation 2) and internal energy (f_E/f; equation 3) components of the elastic force calculated from our data. The enthalpy (that is uncorrected) component of the elastic force is negative at low extensions and becomes increasingly positive as extension increases. It is unlikely that the amorphous chains crystallize with extension because the enthalpy component, which would be negative for such a strain-induced crystallization, becomes positive as extension increases. Rather, the negative enthalpy component at low extensions arises from the thermal-swelling behaviour of the material. When the data are corrected for swelling effects, the internal energy component (f_E/f) becomes a small and essentially constant, positive fraction of the elastic force. The average f_E/f value of 0.14 is similar to that seen for other rubbery materials (Table 1*b*)[13], and indicates that roughly 85% of the retractive force of contracted spider's silk is due to changes in polymer chain conformational entropy.

The thermodynamic properties of contracted dragline silk indicate that the material is a rubber. Apparently, when immersed in water the protein chains in the amorphous regions have sufficient kinetic freedom so that bulk deformations do not cause any major changes in bond energy potentials. Rather, energy is stored primarily as a change in the conformational entropy of these chains. The crystalline regions presumably serve to cross-link the amorphous chains into a network. This proposed structure for contracted (that is, hydrated) silk may shed important light on the behaviour of the silk in its normal, non-contracted state. Protein rubbers in general are rubbery only when swollen in water or some other polar solvent[14]. A lowered water content decreases kinetic freedom of the peptide chains, and the material enters its glass transition, where stiffness increases dramatically, extensibility is reduced, and viscoelastic energy dissipation becomes very important[14]. We propose that the dragline silk of *A. diadematus* in room conditions should be thought of as a rubber that functions near the middle of its glass transition. The amorphous regions provide a reasonably stiff, energy-absorbing matrix within which are embedded very stiff, crystalline inclusions. In this respect silk, like nylon, is a fibre-reinforced composite, and the analysis of silks as composite materials may well explain their unusually high breaking energies[15].

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Inability of Rous sarcoma virus to cause sarcomas in the avian embryo

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The injection of Rous sarcoma virus (RSV) into the wing web of newly hatched chicks causes a rapidly growing sarcomatous tumour which is palpable within 1 week of inoculation[1]; and cultures of fibroblasts derived from chick embryos (CEF) and infected with RSV become rapidly transformed[2]. Genetic studies have determined that expression of a single viral gene, designated v-*src*, is necessary for neoplastic transformation[3]. This gene codes for a 60,000-molecular weight phosphoprotein termed pp60^{src}, which

possesses a protein kinase activity that phosphorylates polypeptides on tyrosine residues and is constitutively expressed in infected CEF cells[4]. It has been suggested that transformation, and possibly tumorigenesis, may result solely from the consequences of this increase in tyrosine phosphorylations[4]. The pathogenicity of RSV in chick embryos *in ovo* is less clear. Murphy and Rous[5] suggested that RSV may have caused tumours in "various tissues" of "some embryos", but the subsequent studies of Milford and Duran-Reynals[6], as well as several other laboratories[7,8], failed to find any evidence of intraembryonic tumours in RSV-infected early embryos. The findings of Duran-Reynals, if correct, cannot be explained easily in view of our present understanding of RSV tumorigenicity. Thus, we have re-examined the interaction of RSV with the avian embryo and confirm here that RSV is non-tumorigenic and non-teratogenic when microinjected into day 4 chicken embryos. In addition, we found that (1) the virus not only replicates in the embryo, but it also expresses an active *src*-specific protein kinase and (2) once the cells from the infected limbs are disrupted and placed in culture, they are capable of expressing the transformed phenotype after a 24-h delay.

Using a capillary tube pulled to 40–75 μm diameter, we inoculated the limb bud of day 4 chicken embryos (stage 22–24)[9] with 10^4–10^5 focus-forming units of Prague-A (PRA) or Schmidt–Ruppin-D (SRD) RSV. Control embryos were inoculated with virus-free medium (medium 199 supplemented with 2% tryptose phosphate broth, 2% fetal calf serum and 1% chick serum—designated medium 2-2-1). Embryos were allowed to continue *in ovo* and eggs were candled daily. Some embryos were killed and analysed at various developmental stages; others were examined at death. We found no appreciable difference between the survival of control and virus-inoculated embryos until 7 days post-injection when the mortality of virus-inoculated embryos increased relative to the inoculated controls, suggesting that events specific to the virus infection had subsequently begun to manifest themselves (data not shown).

To date we have inoculated over 1,500 embryos. Those examined for tissue histology and gross morphology at various times during development showed no indication of neoplastic growth or teratogenesis. Figure 1*d*, *e* shows an example of a limb bud from a 14-day embryo which had been inoculated with virus on day 4. By morphological criteria, chondrogenic and myogenic differentiation are unimpaired. A section through the limb bud of an embryo inoculated with medium alone is presented for comparison (Fig. 1*a*). 25% of the RSV-containing embryos developed blood blisters that varied in size from 1 to 10 mm and resembled the non-neoplastic haemorrhagic lesions first described by Duran-Reynals[6,10] (Fig. 1*b*, *c*). No lesions were observed in control inoculated embryos, nor in embryos infected with transformation-defective (*td*) RSV (not shown).

The absence of response to RSV in embryonic limb buds contrasted sharply with the response in the wing of newborn chicks. As demonstrated by others[1,11], we found that, within 10 days, 75% of the newborn chicks inoculated with 10^4 FFU of RSV has palpable tumours, >5 mm in diameter. Furthermore, as few as 10 FFU generated tumours in 15% of the birds within the same time frame. The histological appearance of haemorrhagic lesions was easily distinguishable from that of RSV-generated tumour tissue taken from a 10-day-old chick inoculated 24 h after hatching (compare Fig. 1*b* and *c* with *f*). The tumour tissue was invasive and contained a heterogeneous population of cells with large irregular nuclei characteristic of neoplastic tissue. As we used cloned viruses and specific pathogen-free (SPF) eggs, we have essentially ruled out artefacts of possible interference by endogenous or exogenous viral contaminants. The results confirm those of Duran-Reynals and others[6,7,10], and suggest that early embryos are indeed refractory to the tumorigenic potential of RSV.

To determine whether or not RSV replicates *in ovo*, control and infected embryos were killed on day 14, and various tissues were homogenized and screened for the presence of reverse transcriptase[12]. We found more than a 10-fold increase in activity of the enzyme in the inoculated limb tissues, and a significant increase of activity in tissues distal to the infected limb bud (Table 1*a*). Virus from these embryos formed up to 2×10^5 FFU per μg of tissue DNA. Tissue isolated from 10-day-old tumours in young chicks contained $0{\cdot}5$–2×10^6 FFU per μg of DNA. It should be pointed out that as we neither observed an obvious tumour nor assayed the entire wing, some cells may not be infected. On the other hand, tissue taken from the tumours in the newborn, by definition, was viraemic. Some virus-inoculated embryos did not show the presence of virus when screened at day 14; we presume that, in these embryos, the injection was unsuccessful.

Despite the absence of tumours, tissues from infected embryos contained elevated levels of viral kinase activity, at times comparable with the levels found in tumour tissue. The level of kinase activity varied among embryos, but in the inoculated limbs there were increases as high as 50-fold compared with control tissues. Other tissues also contained elevated levels of kinase activity, suggesting that the infection had spread throughout the embryo. Elevation of kinase activity was detectable as early as 2 days after inoculation; therefore, full viral gene expression probably occurred soon after infection.

Table 1 Parameters associated with virus production in RSV-infected embryos

a Reverse transcriptase activity

Tissue	Fold increase
Plasma	2.4
Wing	10.1
Breast	3.0
Viscera	1.6
Brain	2.1

b Infectious virus and kinase activity

	Uninfected	Infected	Tumour tissue
FFU $\times 10^{-5}$ per μg DNA	0	1.3 ± 0.8	11.3 ± 57
Kinase per μg DNA	7.5 ± 2.1	190 ± 83	282 ± 57

c Distribution of kinase activity

Tissue	Fold increase
Infected wing	51.00
Liver	2.7
Opposed wing	3.7

Embryos were inoculated as described in the text, killed on day 14 and tissues were immediately frozen in a dry ice bath. *a*, Tissues were subsequently homogenized in 1.5 ml of 10 mM Tris, 1 mM EDTA. 0.05 ml of each tissue specimen was assayed for reverse transcriptase activity according to the procedure of Tereba and Murti[12], and 0.1 ml was assayed for DNA content by the method of Hinegardner[42]. Fold increase is the ratio of reverse transcriptase activity (normalized to DNA) in the tissues of virus-inoculated embryos compared with the normalized reverse transcriptase activity in the corresponding tissue of controls. *b*, Tissues were prepared as in *a* except that, for kinase analysis, they were homogenized in disruption buffer (25 mM HEPES buffer (*p*H 6.8), 1% Nonidet P-40, 0.5 M NaCl, 5 mM EDTA and 2% Trasylol). Kinase assays were done as described[43] except that the reaction buffer was 10 mM HEPES, 5 mM $MnCl_2$, 16 mM NaCl. Each sample was also assayed for DNA content as described[42]. Focus-forming activity was determined as described elsewhere[44] and normalized to the DNA content of the same tissue. Kinase activity was quantified by solubilizing the IgG band, counting the radioactivity and normalizing the counts to the DNA content of the sample. *c*, Fold increase refers to the ratio of kinase activity in tissues infected with virus and normalized to DNA content, compared with the corresponding normalized tissue in controls. Note that despite a 10-fold reduction in FFU, kinase activity at times is as high as in the tumours. As the infected wing cells *in ovo* have 'normal' morphology, they may not be able to replicate the virus at the same rate as transformed cells. This is supported by our preliminary data which indicate that transformed cells plated on normal extracellular matric flatten out and, while they contain appreciable kinase activity, virus replication is reduced considerably[45].

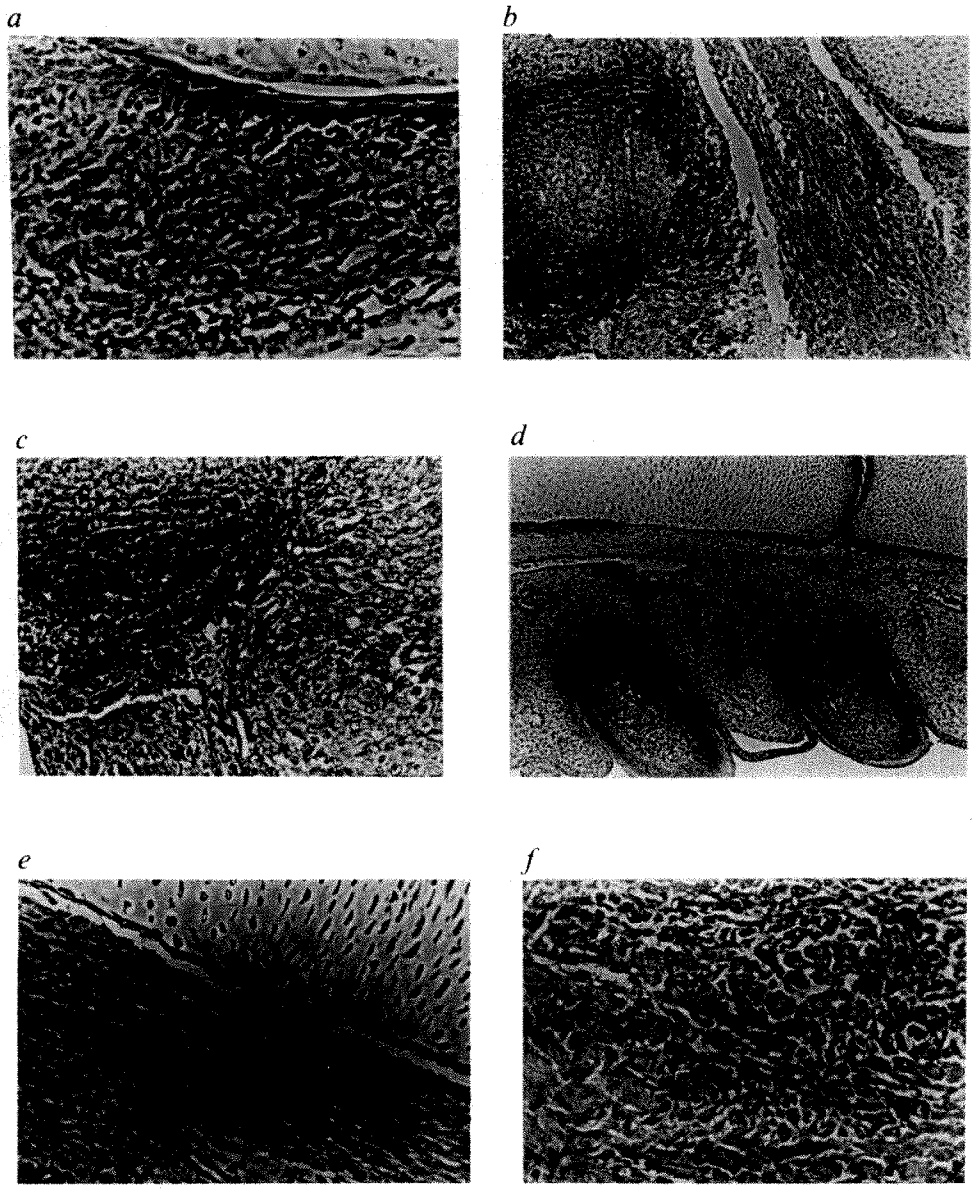


Fig. 1 Histology of inoculated limb buds and tumour tissue. *a–e*, Embryos were inoculated on day 4 as described in the text and were killed on the indicated day. The inoculated limb was removed and fixed in Bouins, embedded in paraffin and sectioned (5 μm). Sections were photographed after staining with haematoxylin and eosin using a Nikon diaphot microscope. *a*, Control limb inoculated with medium alone and killed on day 13. ×280. *b*, Limb inoculated with 10^5 FFU of SRD RSV and killed on day 13. ×105. Note the haemorrhagic lesion on the left. *c*, Same as *b*. Lesion is in the upper left quadrant. ×280. *d*, Limb inoculated with 10^5 FFU of SRD RSV and killed on day 12. ×70. No abnormal morphology was noted. *e*, Same as *d* showing the perichondrium and adjoining tissues. ×280. *f*, Tumour tissue from 10-day-old chicken, inoculated with 10^5 FFU of SRD RSV after hatching. Tumours first appeared on day 7. ×280.

Embryos which failed to show an increase in kinase activity also failed to show reverse transcriptase or focus-forming activity, and probably had not become infected, as discussed above.

We next addressed the following questions: (1) How do the cells from the limbs of infected 14-day-old embryos behave after being disrupted and placed in culture? (2) Are the cultured cells from the limb buds of 4-day-old embryos as susceptible to transformation and virus production as cells from older embryos? Using morphology and the rate of sugar uptake as two commonly measured criteria of transformation, we compared cultures prepared from the wing of inoculated embryos with either cultures derived from the wing of control embryos which were infected at the time of plating, or cultures derived from tumours generated in newly hatched chicks (Fig. 2). Cells were cultured in 25-mm multiwell plates at high density ($4–5 \times 10^5$ cells cm^{-2}) to discourage the horizontal spread of infectious virus. Chick cells cultured in these conditions grow slowly; the percentage of cells infected in the first 72 h is reduced by a factor of 5 or more compared with cultures infected at low density (data not shown). Despite high kinase activity which was elevated in the intact tissue and which remained high after disruption, cells infected *in ovo* appeared quite normal initially, by the criteria of both morphology and rate of sugar uptake. However, within 24 h the cultures became morphologically transformed (*en masse* rather than as foci) and the rate of sugar transport increased. This 'delayed' transformation is not due to the process of tissue disruption associated with the preparation of cultures, as cultures derived from tumour tissue or from fully transformed cultures retained a transformed morphology and high sugar uptake immediately after trypsinization and replating. On the other hand, cultures derived from normal embryos which were infected after plating (multiplicity of infection (MOI) = 0.2; approximately the same virus-to-cell ratio found in the infected limb tissue at the time of culturing) began to show foci of transformation and a stimulation of the rate of sugar transport only after an interval of 72 h.

Thus, embryonic cells infected *in ovo* retain their normal phenotype *in ovo*, as well as initially after plating, but the ability to display a transformed phenotype is rapidly acquired after the cells are placed in culture. It is possible that either the culture conditions are permissive for the expression of the transformed phenotype, or alternatively, these cells may experience an acceleration of the developmental programme which would normally lead to the tumour permissiveness observable in newly hatched chicks. The notion that the developmental programme of chick cells may accelerate in culture at high densities has been suggested by previous work in this laboratory[13].

To determine whether cells from the limb bud of day 4 embryos are as susceptible to transformation, and whether they

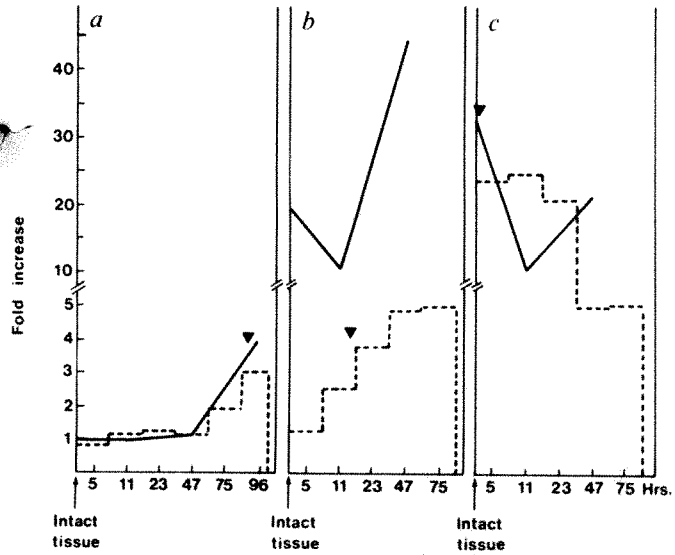


Fig. 2 Determination of transformation in cultures derived from the wing of control embryos infected at the time of plating (*a*), cultures prepared from the wing of inoculated embryos (*b*), and cultures derived from tumours generated in newly hatched chicks (*c*). Embryos were inoculated and killed as described in the text. The inoculated limb was removed and a portion of intact tissue was assayed for kinase activity as described in Table 1. The rest was digested for 45 min at 39 °C in a solution of medium F-10, 0.15% trypsin, 0.3% collagenase (type II) and 5% fetal calf serum. An aliquot was checked under a microscope to determine the extent of digestion and the incubation time was adjusted accordingly. After digestion, the cells were washed twice in medium 2-2-1, and plated at high density (4×10^5 cm^{-2}). At the times indicated, 2-deoxyglucose[46] (broken lines) and kinase[43] (solid lines) assays were performed. The assays for each time point were normalized to that of cultures of cells derived from uninfected embryos and are expressed as the fold increase relative to the control. ▼ Indicates the time of appearance of morphological transformation.

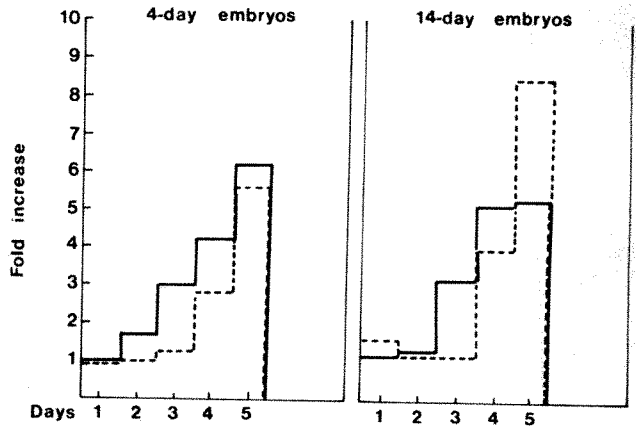


Fig. 3 Susceptibility to transformation of cells from limb buds of day 4 and day 14 embryos. The limb buds from several 4-day-old embryos were microdissected, pooled and dissociated as described in Fig. 2 legend. The lower limb of a 14-day-old embryo was prepared in the same fashion. Both were plated at low density (5×10^4 cells cm^{-2}), and half the plates from each group were infected with SRD RSV at a MOI of 2.0. 2-Deoxyglucose uptake (broken lines) and reverse transcriptase activity (solid lines), as well as morphology and cell number, were monitored daily. Each of the infected cultures was normalized to its uninfected counterparts and the result is expressed as the fold increase compared with the normal control.

produce progeny virus at the same rate, as cells derived from the limbs of day 14 embryos, limb bud tissues from the respective embryos were dissociated, plated at low density and infected with RSV at a high multiplicity of infection (MOI = 2). Cultures were subsequently screened for reverse transcriptase activity, sugar uptake and morphology. Predictably, the growth rates and subsequent morphologies of infected and uninfected cultures derived from day 4 embryos differed from those of their day 14 counterparts. However, the increase in sugar uptake and the production of viral particles (per cell) were comparable in both infected cultures (Fig. 3).

The infection and replication of RSV in certain avian embryo cell types in culture in the absence of transformation has been reported[14,15]. The infected limb cells in our experiments differ somewhat in that they express their transformed phenotype once placed in culture. This is not a selection phenomenon as we have shown recently that 90% of the isolated limb cells are positive for the presence of viral antigens before culturing (D.S.D., M. Gates and M.J.B., unpublished); and as shown in Fig. 2*b*, transformation in these cultures occurs *en masse* within 24 h. Dunkel and Groupe[16] found, several years ago, that embryonic day 3 limb buds infected *in vitro* and grafted onto the chorioallantoic membrane of 9-day-old eggs, show virus replication long before tumour foci appear. The eventual presence of tumours may be due to the disruptive nature of the grafting procedure, the altered developmental environment, or other differences in experimental conditions.

We are presently considering three general hypotheses which might explain the apparent inability of RSV to cause sarcomas in chick embryos. First, the intracellular concentration of viral kinase activity (or its localization) may be suboptimal. Second, a cellular component, required for tumorigenic expression, may be absent from the early embryo; this component might be a target for the viral kinase[17–20], for an 'additional' *src* gene-mediated activity[21–26], or a hormone, growth factor or endogenous promoter[27–30]. In addition to published data[24,25] for cases in which kinase activity alone does not appear to be sufficient for transformation in culture, there are recent data to indicate that some temperature-sensitive mutants of RSV have comparable kinase activity at both permissive and non-permissive temperatures (A. Stoker, P. Enrietto and J. Wyke, personal communication). This indicates the possible importance of other activities and/or domains in pp60^{src} for transformation[21–24]. Third, the early embryo may contain inhibitors for any of these necessary interactions[31,32]. We are presently determining whether or not known targets of pp60^{src} are phosphorylated in tyrosine *in ovo*.

We have been unable to determine whether embryos infected on day 4 of development will ultimately develop tumours. Due to the lethality of the virus around days 12–15, we have hatched only four of these embryos: none contained detectable tumours, although all four died within 4 days of hatching from as yet undetermined causes. Several examples exist both for sustained suppression of the neoplastic potential[31,33,34] and for temporary suppression related to the developmental stage of the animal[16,35–39], but obviously more embryos must be hatched to draw any firm conclusions.

How general are the implications of these findings for retroviral tumorigenicity in the embryo? Clearly, there is target specificity, and the age of the embryo and possibly route of injection are important factors. Smith and Iranyi[40] found evidence for target 'stem' cells in 12-day-old embryos when injected intravenously with myeloblastosis-associated virus (MAV)-2(0). These chickens developed suppression of bursal and thymic lymphoid cell development 2–3 weeks after hatching. A mutant of avian erythroblastosis virus appears to produce erythroblastosis in 12-day-old embryos efficiently, but it does not produce sarcomas in the embryos. Nevertheless, sarcomas are readily produced in 1-week-old chicks by the same mutant[41]. This latter finding supports our general conclusion that production of sarcomas in the embryo is either suppressed or requires additional factors not present in the embryo.

Note that most of the studies with chick embryos have been done using embryos over 10 days old and our studies were done

with 4-day-old embryos. It is possible that the susceptibility of the embryo to RSV tumorigenicity may increase as the embryo develops. It is also possible that if factors from transformed cells were present, one could induce tumours even in 4-day-old embryos. In preliminary studies we have failed to induce tumours with transformed cells. However, we could not ascertain whether or not the cells remained viable and will in future use cells such as quail that could be distinguished from chick cells.

It seems that while kinase activity of $pp60^{src}$ may be necessary for RSV-mediated transformation and tumorigenesis, there are one or several other developmentally related criteria which must be met before expression of the malignant phenotype is possible. The temporal sequence of the expression of viral and cellular factors may also be an important determinant. Infected cells, once placed in culture, overcome the *in ovo* restriction and become transformed rapidly. The mechanisms of both the restriction *in ovo* and transformation in culture are being investigated.

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A relationship between the yeast cell cycle genes *CDC4* and *CDC36* and the *ets* sequence of oncogenic virus E26

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We report here significant primary sequence homology among the predicted translational products of three genes: *CDC4, CDC36* and *ets*. *CDC4* and *CDC36* are *Saccharomyces cerevisiae* cell division cycle genes, while *ets* is a transformation-specific sequence of avian erythroblastosis virus E26. The deduced primary structures of the three gene products were compared by computer to a large data base of known and predicted protein sequences[1]. The search revealed 22.0–25.5% identity over regions of 140–206 codons, respectively between the different pairwise combinations. For these particular sequences, these identity scores fall 3.4–4.0 standard deviations above the empirically-determined mean values of fortuitous similarity. *S. cerevisiae* calls require *CDC36* and *CDC4* in order to complete two early events in the cell cycle: execution of start (*CDC36*)[2] and spindle pole body separation (*CDC4*)[3]. In virus E26, the *ets* sequence is linked in frame with *Δgag* and myb^E in the tripartite structure 5′-*Δgag-myb*^{E}-*ets*-3′, comprising the E26 transforming oncogene[4,5]. The homologies described here suggest that the biochemical functions or regulation of the *CDC4, CDC36* and *ets* products may be related.

Figure 1 shows an alignment of the inferred amino acid sequences of the entire *CDC36* translational product (middle line) with homologous regions of the *CDC4* and *ets* products. The locations of these sequences in their respective genes are shown in Fig. 2. The lengths of the *CDC4, CDC36* and *ets* products are 779, 191 and 491 amino acid residues, respectively. Above the complete 191-residue *CDC36* sequence in Fig. 1 is the 149-residue C-terminal portion of the *CDC4* product. Below *CDC36* is a 206-residue region from the middle of the *ets* product (Fig. 2). The most striking similarities are observed near the *N*-terminus of the *CDC36* product. In this region, between *CDC36* Ala 8 and Ser 22, 6 out of 15 residues match among all three sequences, and 7 out of 15 match between two sequences. Another region of substantial similarity is the 10-residue region from *CDC36* Tyr 39 to Gln 48, in which the *CDC36* product

Table 1 Homology between pairwise combinations of *CDC4, CDC36* and *ets*

Gene pair	% Identity	Standard deviations above mean	Probability of fortuitous match
CDC4–CDC36	25.5	4.0	0.003%
CDC36–ets	22.0	4.0	0.003%
ets–CDC4	22.8	3.4	0.033%

% Identity refers to per cent of matching residues per sequence length. Standard deviations above mean refers to the number of standard deviations the alignment scores fall above a mean value of chance homology empirically determined by aligning a large number of scrambled sequences of identical composition and length (ref. 1 and see text). Probability of fortuitous match refers to expected per cent of random sequences with alignment scores greater than or equal to that of the genuine gene pair alignment, based on an empirical distribution of alignment scores of scrambled sequences (ref. 1 and see text).

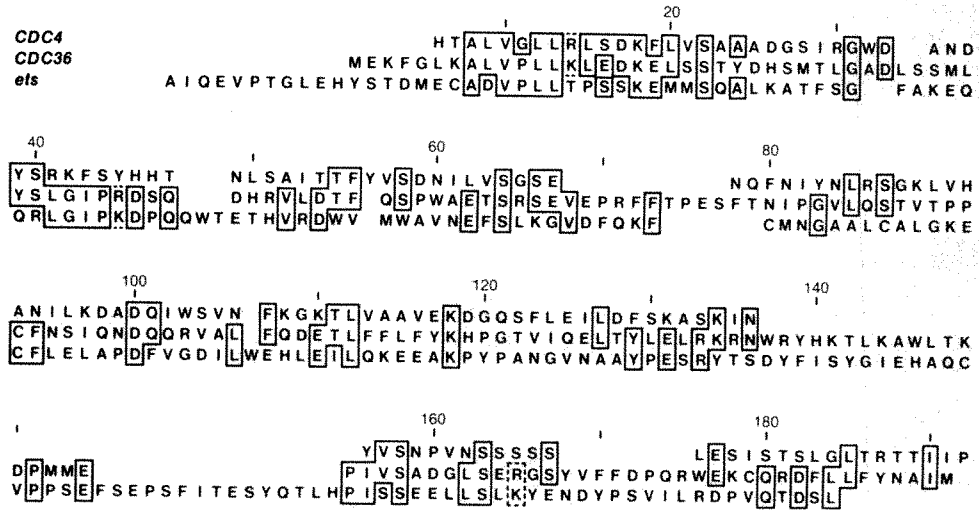


Fig. 1 Alignment of the predicted primary structures of the *CDC4*, *CDC36* and *ets* products. Amino acid identities are boxed; conservative changes are enclosed by dashed boxes. Sequence gaps necessary to obtain optimal alignment are represented by spaces. The coordinates refer to the *CDC36* residue number, with the initiating Met = 1. The *CDC36* product is aligned for maximal homology with the *CDC4* and *ets* products. This alignment does not give the maximal homology between *CDC4* and *ets*. Single-letter abbreviations for the amino acid residues are as follows: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

Methods: *CDC36* and *CDC4* were isolated on recombinant plasmids by transformational complementation (ref. 18 and unpublished data). Each was shown to represent the authentic *CDC36* and *CDC4* genes by their ability to recombine at their respective genomic loci (ref. 18 and unpublished data). The *CDC36* and *CDC4* genes were localized by subcloning, RNA blot analysis and R-loop mapping (ref. 18 and unpublished data). *CDC4* was sequenced by chemical degradation methods[19] and found to contain a large open reading frame of 779 codons. The 149 C-terminal residues of the *CDC4* predicted translational product are shown. Sequencing of *CDC36* by the m13-dideoxy method[20] revealed an open reading frame of 191 codons within the 615-base pair transcribed sequence. The complete *CDC36* deduced translational product is shown. A clone prepared from cDNA transcribed *in vitro* from E26 genomic RNA was sequenced by the m13-dideoxy method[20] and found to contain an *E26 t*ransformation-*s*pecific (*ets*) sequence flanked by *myb*^{E} (an internal 0.8-kb subset of the 1.2-kb AMV-related sequence, *myb*^{A}) and an *env*-related sequence[4]. *ets* encodes the C-terminal 491 amino acid residues of p135 (ref. 4 and Fig. 2). 206 residues of the *ets* predicted translational product are shown.

matches either the *CDC4* or *ets* products at 8 of the 10 positions. We view these similarities as strong evidence that these gene products are related. Overall, the homology between the different pairwise combinations ranges from 22.0 to 25.5%. These scores were compared with the results of a 'jumble test', in which sets of scrambled sequences whose compositions and lengths are identical to those of the two sequences under study (that is, between 149, 191 and 206 codons of *CDC4*, *CDC36* and *ets*, respectively; see Fig. 2) are subjected to the same alignment and scoring procedure used for the authentic alignment[1]. The homology values of the genuine sequences were found to lie 3.4–4.0 standard deviations above the mean values of the randomized sequences, indicating a low probability that the similarities detected are fortuitous (Table 1). We cannot rigorously exclude the possibility that any of the similarities detected here are the results of convergent, rather than divergent, evolution. However, since a particular protein structure can be obtained from many different amino acid sequences, sequence convergence extending over significant lengths (for example, >20 residues) is highly unlikely. Additionally, the requirement for both the *CDC4* and *CDC36* products in the G_1 phase in the same organism (see below) suggests an ancestral relationship for these two genes.

CDC4 and *CDC36* both define early events in the cell cycle of the budding yeast *S. cerevisiae* (see ref. 6 for review). Cells defective in *CDC36* arrest division at start, a point in the late G_1 phase of the cell cycle[2,7]. Start appears to be the cell cycle stage at which cell division is normally controlled, as wild-type cells deprived of essential nutrients or responding to mating pheromone also arrest at start[7]. Cells arrested at this stage are unbudded and have not yet undergone duplication of the spindle pole body, which serves as the microtubule-organizing structure[3]. The next defined step in the mitotic cycle requires *CDC4*. Cells arrested by the *cdc4* defect also fail to initiate DNA synthesis[8] and have duplicated but unseparated spindle pole bodies[3]. Completion of the *cdc4*-sensitive step results in separation of the duplicated spindle pole bodies, which migrate to opposite sides of the nucleus to become the poles of the mitotic spindle[3]. Although arrest by *cdc4* is morphologically advanced with respect to start, cells at both stages remain in G_1. Moreover, both of these arrests share the properties that (1) entry into the S phase requires additional protein synthesis[9] and (2) the cells may enter directly into meiosis without completing a mitotic cycle if transferred to the appropriate medium[10]. By contrast, *CDC7* defines a later period of the G_1 phase which lacks these properties. Therefore, it is not unreasonable to suppose that the structural feature shared by the products of *CDC4* and *CDC36* is essential to their function or regulation in a segment of G_1.

ets is a genomic sequence of the avian erythroblastosis virus E26[4,5]. E26 is related to avian myeloblastosis virus (AMV) in that both viruses are replication-defective, contain a *myb* (or *amv*) sequence, and cause acute myeloblastic leukaemia yet do not transform chicken fibroblasts in culture[11,12]. However, the predominant disease caused by E26 is erythroblastosis, whereas AMV causes myeloblastosis exclusively[13,14]. The E26 oncogene is a tripartite hybrid composed of the in-frame fusions 5′-Δ*gag*-*myb*^{E}-*ets*-3′ (refs 4,5). The Δ*gag*, *myb*^{E} and *ets* sequences comprise 0.8, 0.8 and 1.5 kilobases (kb), respectively, of the hybrid gene[4]. This oncogene codes for a putative transforming protein of 135,000 molecular weight, termed p135 (ref. 12), of which *ets* encodes the C-terminal 491 residues[4] (Fig. 2). DNA hybridization experiments revealed the existence of cellular proto-*ets* sequences, which are unlinked to proto-*myb*[4,5].

The biological functions of the *CDC4*, *CDC36* and *ets* products remain unknown. The product of another yeast start gene, *CDC28*, is homologous to the products of the *src* gene family, many of which have been shown to encode protein kinases[15]. In view of these results it is conceivable that the products of retroviral transforming genes share functional domains with proteins that control cell division. Other authors have reported

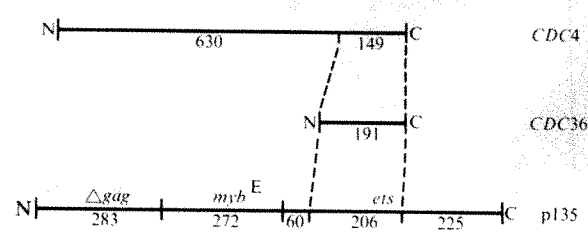


Fig. 2 The locations of the sequences compared in Fig. 1 relative to the complete gene products. Numbers below each line indicate the residue length of that section. Dashed lines enclose homologous sequences presented in Fig. 1. N and C refer to the amino and carboxy termini, respectively. Drawn approximately to scale.

significant homology between other yeast sequences and the vertebrate oncogenic sequence *ras*[16,17]. Our findings extend these observations and support the notion that the mechanisms controlling cell division in yeast and cellular proliferation in vertebrates may be related.

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A novel protease from yeast with specificity towards paired basic residues

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Paired basic residues have been observed as sites of proteolytic processing of prohormones in a wide range of eukaryotic species[1–3]. This strongly suggests that proteases exhibiting specificity towards paired basic residues may be involved in prohormone processing, but candidate enzymes have not so far been identified. Yeast *Saccharomyces cerevisiae* α-cells synthesize and secrete α-mating factor, a peptide of 13 amino acids[4,5], the processing of which from a larger precursor involves cleavage at paired basic residues (-Lys-Arg-)[3,6]. We have therefore used them as a simple model system for the study of prohormone processing and report here the identification, in cell lysates, of a novel protease which specifically recognizes and cleaves the peptide bonds between consecutive basic residues. The purified enzyme, which we have called propheromone-convertase Y, has a molecular weight (MW) of around 43,000. It cleaves various peptide substrates at paired basic residues, but not at single basic residues, implying it is distinct from trypsin-like proteases. Its unique substrate specificity suggests the enzyme may be involved in propheromone processing *in vivo*.

Proteolytic activity specific to consecutive basic residues was monitored in lysates of yeast *S. cerevisiae* α-cells by measuring in a sensitive radioimmunoassay[7] the amount of Tyr-Gly-Gly-Phe-Leu-Arg ([Leu]enkephalin-Arg) released from the synthetic C-terminally α-amidated peptide Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Phe-Ala-NH_2 ([Leu]enkephalin-Arg-Arg-Phe-Ala-NH_2). Since the antiserum used in the radioimmunoassay was directed against the C-terminus of [Leu]enkephalin-Arg, neither the substrate nor its fragmented peptides other than [Leu]enkephalin-Arg showed immunoreactivity[7].

The soluble fraction of the yeast lysates was chromatographed on a column of DEAE-Sephadex A-25. The enzyme activity

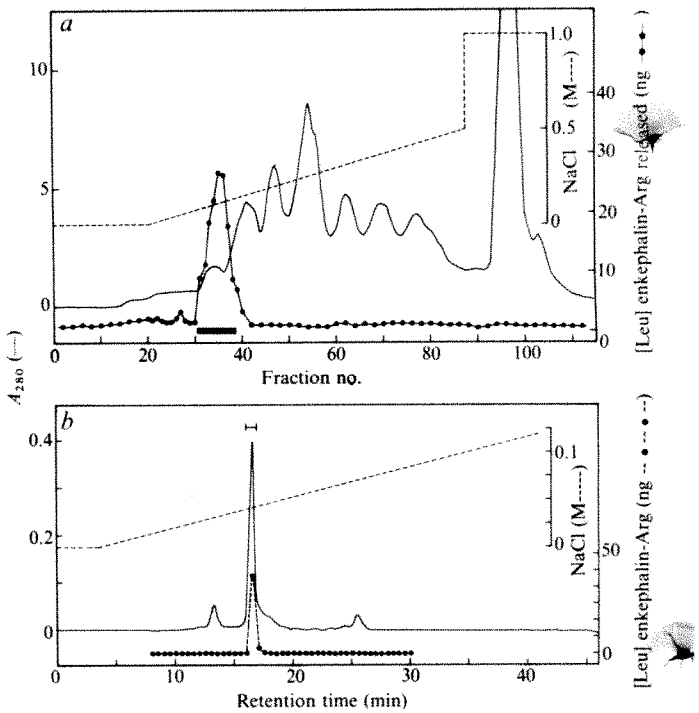


Fig. 1 Purification of the enzyme from lysates of yeast α-cells. *a*, DEAE-Sephadex A-25 chromatography of crude extracts of yeast α-cells. *b*, Final purification of the enzyme by second FPLC on a Mono Q anion-exchange column.
Methods: Haploid strain *S. cerevisiae* X-2180 1B (α-mating type cell) was supplied by Dr T. Tanaka, Suntory Ltd. For purification of the enzyme, cells were grown aerobically at 28 °C for 50 h in 6 l of YM-medium, containing 0.3% yeast extract, 0.3% malt extract, 0.5% polypeptone (pancreatic digest of casein) and 1% glucose. Cells (wet weight 45 g) were lysed by incubation with Zymolyase-60,000 (Seikagaku Kogyo) at 35 °C for 4 h (ref. 17), and homogenized with a Polytron homogenizer. After centrifugation at 30,000*g* for 30 min, ammonium sulphate was directly added to the supernatant to a final concentration of 90% saturation and the resulting precipitate was resuspended in 10 mM Tris-HCl buffer, *p*H 8.0, and dialysed against the same buffer. The dialysate was applied to a column of DEAE-Sephadex A-25 (3 × 30 cm), equilibrated with the same buffer. The column was washed with 10 mM Tris-HCl buffer (*p*H 8.0) and then eluted with a 1-l linear gradient from 0 to 0.5 M NaCl in the same buffer. Fractions of 15 ml were collected at a flow rate of 30 ml h^{-1}. Enzyme activity was assayed by using the synthetic enkephalin-containing peptide, Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Phe-Ala-NH_2, as a substrate. Aliquots (20 μl) from each fraction were incubated at 37 °C for 2 h with 0.1 nmol of the substrate in 100 μl of 0.1 M ammonium acetate buffer (*p*H 5.5) containing 1 mM EDTA and 1 mM dithiothreitol. After stopping the reaction by 10 min of boiling, the amount of [Leu]enkephalin-Arg generated was measured by radioimmunoassay using an antiserum directed against the C-terminus of [Leu]enkephalin-Arg as reported previously[7] (–●–●–●–). The enzyme fractions (marked with bar) obtained from DEAE-Sephadex A-25 chromatography were pooled and concentrated by 90% ammonium sulphate precipitation. The precipitate was dissolved in 8 ml of 0.1 M ammonium acetate buffer (*p*H 5.5) containing 1 mM EDTA and applied to a column (3 × 140 cm) of Sephacryl S-300 (superfine), equilibrated with the same buffer. The enzyme activity was assayed as described above and the active fractions eluted in 660–740 ml were pooled and dialysed against 5 mM Tris-HCl buffer, *p*H 8.0. The dialysate was subjected to FPLC using an anion-exchange Mono Q column (0.5 × 5 cm, Pharmacia). Elution was carried out with a linear gradient from 0 to 0.125 M NaCl in 5 mM Tris-HCl buffer (*p*H 8.0). The main active fractions were collected and diluted with 5 vols of distilled water and rechromatographed in the same conditions as described above. The effluent was monitored by absorbance at 280 nm. Fractions of 0.5 ml were collected at a flow rate of 1.0 ml min^{-1}. Aliquots (0.2 μl) of each fraction were assayed by 2 h of incubation with 0.1 nmol of substrate ([Leu]enkephalin-Arg-Arg-Phe-Ala-NH_2) in 100 μl of 0.1 M Tris-HCl buffer, *p*H 7.4 (--●--●--●--). The active fraction (marked with bar) was collected and used as the purified enzyme.

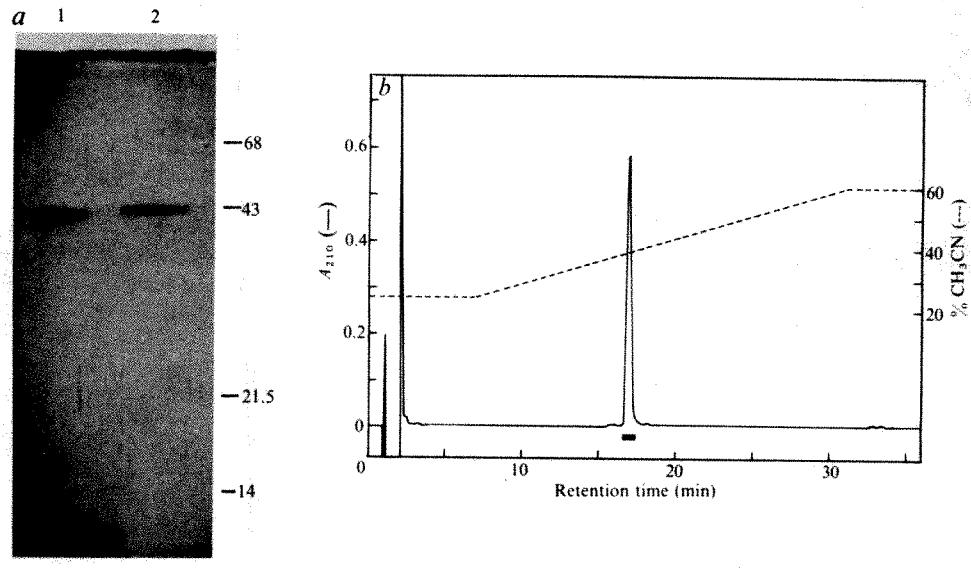


Fig. 2 *a*, SDS-polycrylamide gel electrophoresis analyses of the purified enzyme in the absence (lane 1) or the presence (lane 2) of the reducing reagent. The purified enzyme obtained from Mono Q chromatography in Fig. 1*b* was loaded on 2.0-mm slab gel using a 15% polyacrylamide gel and a discontinuous buffer system[18]. Proteins were analysed using silver stain method (Bio Rad). Lane 1, 100 ng of the unreduced enzyme. Lane 2, 100 ng of the reduced enzyme, treated with 5% 2-mercaptoethanol at 100 °C for 3 min before application to the gel. The R_F positions of molecular weight standards (bovine serum albumin, MW 68,000; ovalbumin, MW 43,000; soybean trypsin inhibitor, MW 21,500; cytochrome *c*, MW 14,000) are indicated at the right. *b*, Reverse-phase HPLC analysis of the purified enzyme. One eighth of the purified enzyme obtained from Mono Q chromatography in Fig. 1*b* was analysed by reverse-phase HPLC on a column (0.4 × 25 cm) of TSK LS-410 ODS-SIL (Toyosoda). Elution was carried out with a linear gradient (24 min) of CG_3CN concentration from 24 to 60% in 0.1% trifluoroacetate solution. Flow rate was 2 ml min^{-1}. The effluent was monitored by absorbance at 210 nm.

producing [Leu]enkephalin-Arg from the substrate was found in fractions 31–38, which were well separated from other proteins (Fig. 1*a*). The active portion was further purified by gel-filtration on a Sephacryl S-300 column, followed by ion-exchange fast protein liquid chromatography (FPLC) on a Mono Q column and finally a second FPLC on the same column (Fig. 1*b*), which resolved the enzyme activity as a single protein peak. The purified enzyme was verified to be homogeneous on SDS-polyacrylamide gel electrophoresis both in the absence and in the presence of 5% 2-mercaptoethanol (Fig. 2*a*), from which we conclude it consists of a single polypeptide chain with a MW of about 43,000, and by analysis on reverse-phase HPLC (Fig. 2*b*). Although HPLC resulted in a marked decrease of enzyme activity, an appreciable amount of enzyme activity (indicated by bar) was detected only at the position of a single symmetrical protein peak. Starting with 45 g of yeast α-cells, the procedure yielded about 0.1 mg of purified enzyme.

HPLC of reaction products from [Leu]enkephalin-Arg-Arg-Phe-Ala-NH_2 after 2 h of incubation with the purified enzyme at 37 °C resolved three peptide peaks (1–3), which, on the basis of amino acid analyses and comparison of retention time on HPLC with authentic peptides, have been identified as Arg-Phe-Ala-NH_2 (peak 1), Tyr-Gly-Gly-Phe-Leu-Arg (peak 2) and the starting Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Phe-Ala-NH_2 (peak 3). The stoichiometric recovery of reaction products (peak 1, 3.7 nmol; peak 2, 3.6 nmol) suggests that the enzyme exclusively hydrolyses the Arg-Arg bond of the substrate. To characterize the substrate specificity of the enzyme, various peptides were incubated with it and the resultant mixtures analysed on reverse-phase HPLC. We found the enzyme converts [Leu]enkephalin-Lys-Arg-Phe-Ala-NH_2 to [Leu]enkephalin-Lys and Arg-Phe-Ala-NH_2, and [Leu]enkephalin-Arg-Lys-Tyr-Pro-NH_2 to [Leu]enkephalin-Arg and Lys-Tyr-Pro-NH_2, by cleaving the Lys-Arg bond and the Arg-Lys bond respectively (Fig. 3*b*, *c*). It catalysed the hydrolysis of X—Y peptide bonds (X, Y: basic residues) of various peptides examined, but peptides containing a single basic residue in the molecule, such as [Leu]enkephalin-Arg-NH_2, [Met]enkephalin-Arg-Phe-NH_2 (Fig. 3*d*) and [Met]enkephalin-Arg-Gly-Leu, were resistant to the action of the enzyme. Furthermore, typical substrates for trypsin, such as tosyl-L-arginine methyl ester (Tos-Arg-OMe; concentration 1.04 mM)[8] and benzoyl-L-arginine 4-methyl-coumaryl-7-amide (Bz-Arg-MCA; 50 μM)[9], were not affected by the enzyme. When rimorphin, which has both consecutive basic residues (-Arg-Arg-) and a solitary basic residue (-Lys-) in the molecule[10] was examined as a substrate, only the Arg-Arg peptide bond was split by the enzyme (Fig. 3*e*, Table 1). These results strongly suggest that the enzyme is an endopeptidase, which specifically attacks the peptide bond between consecutive basic residues. Thus its action is distinct from that of trypsin.

The kinetic constants for [Leu]enkephalin-Arg-Arg-Phe-Ala-NH_2 and [Leu]enkephalin-Lys-Arg-Phe-Ala-NH_2 were determined from Lineweaver–Burk plots with substrate concentrations varying from 0.2 to 1.6 mM. Each substrate was incubated with the enzyme ($[E_0] = 1.09 \times 10^{-8}$ M) for 10 min at 37 °C in 100 μl of Tris-HCl buffer, *p*H 7.4. The reaction was stopped by the addition of 100 μl of 1 M HCl and the amounts of Arg-Phe-Ala-NH_2 released were analysed by amino acid analyses after separating the peptides on reverse-phase HPLC. The apparent K_m and k_{cat} values thus determined were 0.588 mM and 6.82 s^{-1} for [Leu]enkephalin-Arg-Arg-Phe-Ala-NH_2 and 0.329 mM and 2.52 s^{-1} for [Leu]enkephalin-Lys-Arg-Phe-Ala-NH_2 respectively.

The effects of *p*H and various inhibitors on the activity of the enzyme were examined, using [Leu]enkephalin-Arg-Arg-Phe-Ala-NH_2 as a substrate. The optimal *p*H for enzyme activity was around 7.5, with considerable activity being observed in the wide *p*H range 5–10. The enzyme was completely inhibited by serine-protease inhibitors such as phenylmethylsulphonyl fluoride and diisopropyl fluorophosphate at concentrations of

Table 1 Substrate specificity of the enzyme, with cleavage sites indicated by arrows

Peptides hydrolysed by the enzyme:

Tyr-Gly-Gly-Phe-Leu-Arg↓Arg-Phe-Ala-NH_2

Tyr-Gly-Gly-Phe-Leu-Lys↓Arg-Phe-Ala-NH_2

Tyr-Gly-Gly-Phe-Leu-Arg↓Lys-Tyr-Pro-NH_2 (β-neoendorphin-NH_2)

Tyr-Gly-Gly-Phe-Leu-Arg↓Arg-Ile (PH-8P)

Asp-Tyr-Gln-Lys↓Arg-Tyr-Gly-Gly-Phe-Leu

Tyr-Gly-Gly-Phe-Leu-Arg↓Arg-Gln-Phe-Lys-Val-Val-Thr (rimorphin)

Peptides not hydrolysed by the enzyme:
Tyr-Gly-Gly-Phe-Leu-Arg, Tyr-Gly-Gly-Phe-Leu-Lys,
Tyr-Gly-Gly-Phe-Leu-Arg-NH_2, Tyr-Gly-Gly-Phe-Met-Arg-Phe,
Tyr-Gly-Gly-Phe-Met-Arg-Phe-NH_2, Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu,
Tos-Arg-OMe(TAME), Bz-Arg-MCA

Each peptide (~4 nmol) was incubated with the enzyme in the same conditions as described in Fig. 3 legend. Reaction mixtures were analysed on reverse-phase HPLC and the resultant peptide peaks identified by amino acid analyses. Basic amino acid residues are underlined.

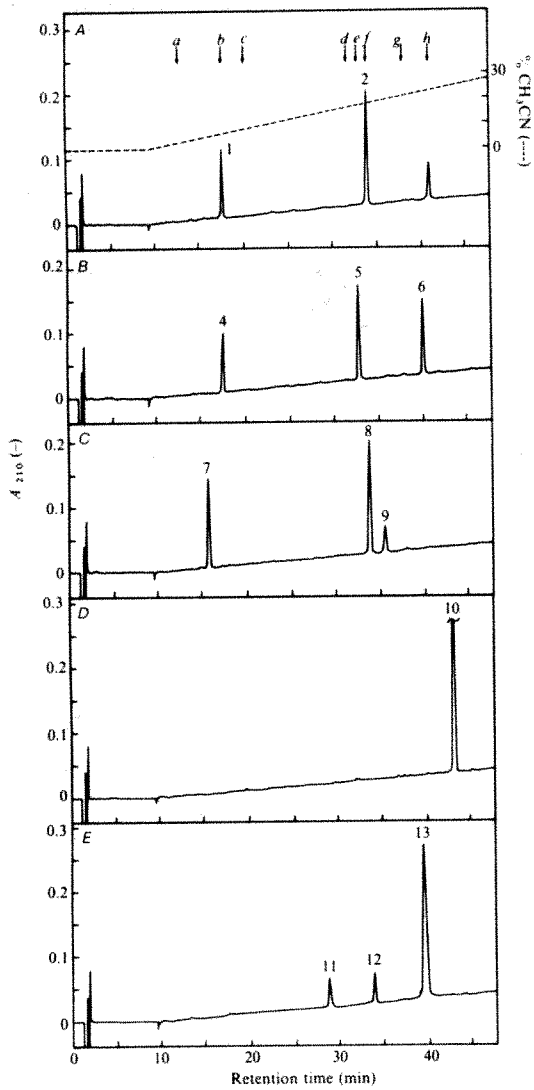


Fig. 3 HPLC patterns of the reaction products from [Leu]enkephalin-Arg-Arg-Phe-Ala-NH_2 (*A*), [Leu]enkephalin-Lys-Arg-Phe-Ala-NH_2 (*B*), [Leu]enkephalin-Arg-Lys-Tyr-Pro-NH_2 (*C*), [Met]enkephalin-Arg-Phe-NH_2 (*D*) and rimorphin (*E*), obtained by incubation with the purified enzyme. Approximately 4 nmol of the substrate peptides were incubated at 37 °C for 2 h with 0.1 μg of the purified enzyme in 100 μl of Tris-HCl buffer, *p*H 7.4. After the reaction was stopped by the addition of 20 μl of 1 M HCl, the solution was directly applied to a column of TSK LS-410 ODS-SIL (0.4 × 25 cm). Elution was carried out with a linear gradient (80 min) of CH_3CN concentration from 0 to 60% in 0.1% trifluoroacetate solution. Flow rate was 2 ml min^{-1}. The effluent was monitored by absorbance at 210 nm. The respective peptide peaks were collected, hydrolysed with 6 M HCl at 110 °C for 22 h, and analysed on an amino acid analyser (Hitachi 835). Based on amino acid composition and comparison of retention time with authentic peptides, the peptide peaks were identified as follows (yields of the peptides are designated in parentheses). *A*: 1, Arg-Phe-Ala-NH_2 (3.7 nmol); 2, Tyr-Gly-Gly-Phe-Leu-Arg (3.6 nmol); 3, Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Phe-Ala-NH_2 (0.7 nmol). *B*: 4, Arg-Phe-Ala-NH_2 (3.1 nmol); 5, Tyr-Gly-Gly-Phe-Leu-Lys (2.9 nmol); 6, Tyr-Gly-Gly-Phe-Leu-Lys-Arg-Phe-Ala-NH_2 (1.4 nmol). *C*: 7, Lys-Tyr-Pro-NH_2 (3.5 nmol); 8, Tyr-Gly-Gly-Phe-Leu-Arg (3.4 nmol); 9, Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-NH_2 (0.3 nmol). *D*: 10, Tyr-Gly-Gly-Phe-Met-Arg-Phe-NH_2 (3.9 nmol). *E*: 11, Arg-Gln-Phe-Lys-Val-Val-Thr (1.0 nmol); 12, Tyr-Gly-Gly-Phe-Leu-Arg (0.98 nmol); 13, Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr (2.7 nmol). The elution positions of authentic peptides are indicated by arrows: *a*, Phe-Ala-NH_2; *b*, Arg-Phe-Ala-NH_2; *c*, Arg-Arg-Phe-Ala-NH_2; *d*, Tyr-Gly-Gly-Phe-Leu-Lys-Arg; *e*, Tyr-Gly-Gly-Phe-Leu-Lys; *f*, Tyr-Gly-Gly-Phe-Leu-Arg; *g*, Tyr-Gly-Gly-Phe-Leu; *h*, Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Phe-Ala-NH_2.

2×10^{-4} M. Thiol-protease inhibitors, such as monoiodoacetate, and *p*-chloromercuribenzoic acid and metal chelators, such as EDTA and 1,10-phenanthroline at 10^{-3} M, had no effect on enzyme activity. The enzyme can therefore be classified as a serine protease. However, its resistance to the general trypsin inhibitors tosyl-L-lysine chloromethyl ketone (10^{-3} M) and leupeptin (10^{-4} M) indicate that it is distinct from pancreatic trypsin and other related proteases. The recognition mechanism of paired basic residues exhibited by the enzyme was very different from that exhibited by trypsin.

The enzyme described here appears to be a unique and novel endoprotease, showing a substrate specificity and an inhibitor spectrum different from other putative prohormone-processing enzymes[11–16]. The strict substrate specificity of the enzyme towards paired basic residues indicates that the enzyme may be physiologically involved in the essential function of propheromone processing. Further investigation is necessary to demonstrate whether the cleavage at the peptide bond between the paired basic residues generally occurs as the initial proteolytic event in prohormone-processing in tissues.

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Lithium reversibly inhibits microtubule-based motility in sperm flagella

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The sliding-tubule hypothesis of flagellar movement has strong experimental support[1], but our present knowledge of the mechanism by which this sliding is coordinated and converted into flagellar oscillation and bend propagation is quite limited. Among the few facts known are: (1) calcium has a role in regulating the asymmetry of flagellar waveform[2,3], (2) the initiation or activation of motility in spermatozoa from several species involves a cyclic AMP-dependent phosphorylation reaction[4–7], and (3) the conversion of tubule sliding to bending does not require the radial spokes or central tubules[8,9]. Inhibitors are valuable tools for investigating mechanisms involved in cellular function, and we report here that Li^+ in low concentrations reversibly inhibits the microtubule-based movement of reactivated sea urchin sperm flagella. The evidence indicates that the action of Li^+ is directed primarily towards one or more regulatory sites through which Ca^{2+} modulates the asymmetry of flagellar waveform, rather than towards dynein ATPase

itself. Lithium also appears to inhibit the sperm adenylate cyclase, but this action does not seem to be relevant to its inhibition of normal motility. Our findings indicate the need for considerable caution when using ATP analogues supplied as the Li^+ salt.

Sperm from the sea urchin, *Tripneustes gratilla*, were demembranated in the presence of Ca^{2+} (refs 2, 3) and reactivated at 22–23 °C (Fig. 1). In these conditions the waveforms of the sperm flagella are nearly symmetrical when they are reactivated with Mg-ATP^{2-} at low ($<10^{-6}$ M) Ca^{2+} concentration, becoming asymmetrical only at higher Ca^{2+} concentrations. In the reactivating solution used here, the Mg-ATP^{2-} concentration is 0.5 mM, free Mg^{2+} is 0.1 mM (ref. 10), and the sperm flagella beat at a frequency of 26.0 ± 1.1 Hz (average of six preparations, 69 sperm). Addition of 6–8 mM Li acetate caused abrupt and complete cessation of movement, with the majority of the flagella assuming a straight or almost straight configuration. Dilution of these immobilized sperm 100-fold into fresh reactivating solution without Li^+ immediately restored movement in all of the sperm, with the flagellar beat frequencies and waveforms being indistinguishable from those in the starting preparation. A few experiments were also performed with sperm prepared by demembranation in a solution containing 2 mM EGTA and no added Ca^{2+} (refs 2, 3). When these sperm were reactivated with 0.5 mM Mg-ATP^{2-}, their flagellar waveforms were markedly asymmetrical, even in reactivating solution with a low concentration of Ca^{2+} ($<10^{-8}$ M), and addition of 0.1 mM Ca^{2+} induced quiescence, with the sperm flagella assuming a stationary, sharply bent cane-shape[3]. Addition of Li^+ to such preparations at low Ca^{2+} concentration inhibits the motility similarly to that of sperm demembranated in the presence of Ca^{2+}, except that instead of being nearly straight, the immobilized flagella are bent into cane-shapes resembling the quiescent form produced by Ca^{2+} (refs 3, 11). Addition of 5 mM LiCl to live sperm swimming in seawater had no noticeable effect on motility parameters, but 55 mM LiCl increased waveform asymmetry significantly and decreased longevity.

The standard reactivating solution for this work contained less Mg^{2+} than in our earlier studies because free Mg^{2+} seems to compete with Li^+. Thus, while 6 mM Li^+ was sufficient to inhibit the sperm in the presence of 0.1 mM free Mg^{2+}, as much as 20 mM Li^+ was needed at 3 mM free Mg^{2+}. Moreover, addition of either 3 mM Mg^{2+} or 6 mM Ca^{2+}, to sperm inhibited initially by 6 mM Li^+ at 0.1 mM free Mg^{2+}, induced the resumption of motility in >90% of the sperm.

Li^+ at concentrations lower than are needed to completely stop the reactivated movement cause a decrease in flagellar beat frequency (Fig. 1). As the Li^+ concentration is increased, a substantial increase in the asymmetry of the bending waves is observed, and a progressively greater percentage of the sperm become non-motile. Although it becomes difficult to strobe the highly asymmetric sperm flagella above 4 mM Li^+, it appears that none beat at frequencies lower than ~17 Hz, corresponding to 65% of the uninhibited value.

The demembranated sperm can be desensitized to the inhibitory effects of Li^+ by brief digestion with trypsin at a trypsin/sperm protein ratio of ~1:10. Addition of 0.2 μg trypsin ml^{-1} to the reactivating solution containing sperm immobilized with 6–24 mM Li^+ induced a gradual resumption of flagellar beating until after 2–3 min over 90% of the sperm were swimming vigorously. Their waveforms were symmetric and resembled those in undigested control preparations without Li^+, but their beat frequency was diminished to an extent that depended on the Li^+ concentration (Fig. 2). The rate at which motility was restored by trypsin is approximately the same as the rate at which trypsin eliminated the Ca^{2+}-induced asymmetry of flagellar waveform in a parallel observation dish containing 6 mM Ca^{2+} and no Li^+ (ref. 12).

Li^+ is known to inhibit many adenylate cyclases[13,14], and our preliminary experiments have suggested that the cyclase in these sperm is also blocked by Li^+. In view of this fact, we investigated the effect of cyclic AMP on Li^+ inhibition and found that addition of 10 μM cyclic AMP to preparations inhibited by

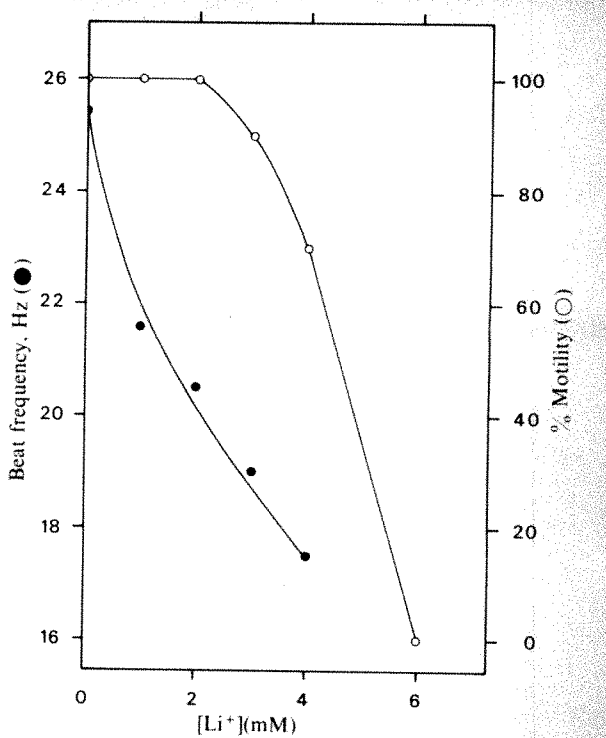


Fig. 1 Decrease in beat frequency (●) and percentage of motile sperm (○) as a function of Li^+ concentration. Sperm were demembranated in a solution containing 0.04% Triton X-100, 4 mM $CaCl_2$, 1 mM dithiothreitol (DTT), 0.1 mM EGTA and 10 mM Tris-HCl, *p*H 8.0 (potentially symmetrical conditions)[3] and reactivated at 21.5 °C in a solution containing 0.2 M K acetate, 0.1 mM EGTA, 1 mM DTT, 2% polyethylene glycol (molecular weight 20,000), 0.6 mM $MgSO_4$, 1.0 mM ATP, 10 mM Tris-HCl, *p*H 8.3 (ref. 22), and the indicated concentration of Li acetate.

6 mM Li^+ caused a partial reversal of the inhibition. In most of the 21 preparations observed, 30–70% of the sperm flagella resumed beating with highly asymmetric waveforms, although in a few it was as low as 15% or as high as 90%. The average frequency in these conditions was 18.4 ± 1.4 Hz (4 preparations, 54 sperm), significantly lower than that of sperm flagella desensitized by trypsin digestion, at this Li^+ concentration (Fig. 2). It is unclear what factors influence the degree of reversal obtained with cyclic AMP. Increasing the cyclic AMP concentration to 100 μM had no further effect. However, increasing the Li^+ concentration to 16 mM always resulted in a substantial decrease in the extent of reversal obtained with cyclic AMP, although it did not affect the extent of reversal obtained by dilution; in one such experiment, 25–30% of the sperm resumed swimming in 8 mM Li^+ after addition of 10 μM cyclic AMP, whereas only 0–5% regained motility in 16 mM Li^+. Addition of 10 μM cyclic GMP instead of cyclic AMP did not reverse the Li^+ inhibition. Also, addition of 10 μM cyclic AMP to control sperm reactivated without Li^+ had no noticeable effect on either the waveforms or frequency in the conditions tested.

To determine whether motility depends on the continuing synthesis of cyclic AMP by adenylate cyclase in normal preparations and whether the inhibition of motility by Li^+ is linked to inhibition of this activity, we replaced the ATP in our reactivating solution with analogues lacking a 3′-hydroxyl group that cannot serve as substrates for adenylate cyclase. Both 3′-deoxy ATP and 2′,3′-dideoxy ATP (at 1.0 mM) induce flagellar beating at about 13 Hz and 9 Hz, respectively, with waveforms and longevity very similar to those obtained with ATP. Moreover, this reactivated motility is inhibited by Li^+, although concentrations of 10–20 mM are required, suggesting that the affinity of these analogues for Mg^{2+}, Li^+, or for dynein ATPase is different from that of ATP. It seems, therefore, that while Li^+ may block adenylate cyclase activity, this is not the mechanism by which it inhibits flagellar motility.

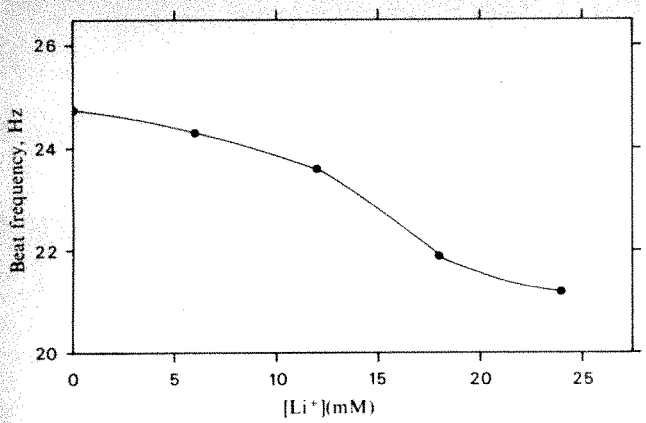


Fig. 2 Decrease in beat frequency as a function of Li^+ concentration of sperm restored to motility by trypsin digestion. Sperm were demembranated and reactivated as described in Fig. 1 legend, then inhibited by addition of Li^+ from 0 to 24 mM, followed by addition of 0.2 μg ml^{-1} trypsin. Beat frequencies were read ~2–3 min later, when the flagellar beating most closely resembled that of normal waves. Beyond this period, the movement began to deteriorate until finally the sperm stopped at ~5 min.

Our overall data thus favour a mechanism in which cyclic AMP increases the threshold concentration for Li^+ inhibition of motility, possibly through changing the activation level of the flagellum, while having little effect on the concomitant Li^+-induced reduction in beat frequency and increase in waveform asymmetry.

Further experiments were designed to test whether Li^+ inhibits dynein arm cross-bridge cycling or ATPase activity. First, we investigated the effect of Li^+ on sperm flagella set into rigor bends in reactivating solution lacking ATP[15], and found that the presence of Li^+ up to 80 mM did not block the straightening of rigor waves that occurred on subsequent addition of 1 mM ATP. Second, we measured the effect of Li^+ on the rate of hydrolysis of Mg-ATP^{2-} by solubilized latent activity dynein 1, and found that it decreased by only 15% in the presence of 4–64 mM Li acetate. On the other hand, the rates of Mg-ATP^{2-} hydrolysis for motile and non-motile (homogenized) preparations of reactivated sperm[16] were decreased from 0.14 ± 0.01 and 0.035 ± 0.005 μmole P_i per min mg protein, respectively, to 0.017 ± 0.003 and 0.013 ± 0.004 μmol P_i per min mg protein in the presence of 10 mM Li^+ in 0.2 M K acetate at *p*H 8.2. The Li^+-induced decrease in ATPase activity of motile sperm preparations presumably reflects a loss of movement-coupled ATPase activity resulting directly from immobilization of the sperm flagella.

Our results indicate Li^+ does not inhibit dynein arm cycling *per se*, but rather interacts with a regulatory mechanism—this is shown most clearly by the fact that brief digestion desensitizes the reactivated sperm to inhibition by Li^+. As low concentrations of Li^+ mimic the effects of Ca^{2+} in inducing asymmetrical beating and quiescent-shaped waveforms, it seems likely that at least one of the regulatory sites concerned is that which normally modulates the Ca^{2+}-dependent asymmetry of flagellar waveform. Besides Ca^{2+} (ref. 3) and Li^+, the only agents known to inhibit flagellar motility without inhibiting dynein arm cycling activity are CO_2 (ref. 17) and low *p*H[18]. The targets for these agents are unknown, but CO_2 appears to act through an amplitude-regulating mechanism.

The depression of beat frequency (0–13%) that follows desensitization by trypsin in Li^+ concentrations ranging from 0 to 24 mM probably results from a decreased Mg-ATP^{2-} concentration as a consequence of the formation of Li-ATP^{3-}. We calculate that with 6 mM Li^+ added to our reactivating solution, the concentrations of Mg-ATP^{2-} and Li-ATP^{3-} become 0.45 and 0.14 mM, respectively[19], and by direct measurement we have found that Li-ATP^{3-} is not hydrolysed by soluble dynein 1 at a significant rate. However, our present data do not allow us to say whether Li^+ or Li-ATP^{3-} is the primary form responsible for the increased asymmetry and inhibition of motility in non-trypsin-treated sperm. The apparent competition of free Mg^{2+} or Ca^{2+} with Li^+ could be due to competition for complexing with ATP. However, because many of its effects resemble those of Ca^{2+}, we postulate that Li^+ may be acting as an analogue of Ca^{2+} at a regulatory site that binds Ca^{2+} at elevated Ca^{2+} concentrations, and Mg^{2+} at low Ca^{2+} concentration[20]. Okuno and Brokaw reported similar difficulty in determining whether Ca^{2+} or Ca-ATP^{3-} interacts with the axoneme to induce either asymmetrical beating, or non-oscillatory beating in the presence of vanadate[21].

Our work suggests that further study of the effects of Li^+ on cyclic AMP- and Ca^{2+}-dependent regulatory steps may have broad usefulness in unravelling the complex interactions involved in the control of flagellar motility and other cellular functions.

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Rapid mobilization of Ca^{2+} from rat insulinoma microsomes by inositol-1,4,5-trisphosphate

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Several hormones and neurotransmitters raise the cytosolic free Ca^{2+} concentration by stimulating the influx of Ca^{2+} and/or by mobilizing stored Ca^{2+} (refs 1, 2). However, the link between the agonist receptor on the cell surface and the organelle(s) from which Ca^{2+} is mobilized is unknown. One feature of the agonists that increase cytosolic Ca^{2+} is their rapid induction of phosphatidylinositol turnover[3–8] and polyphosphoinositide hydrolysis[9–14]; in some tissues this leads, within seconds, to a marked accumulation of the water-soluble products, inositol 1,4-bisphosphate (Ins1,4P_2) and inositol-1,4,5-trisphosphate (Ins1,4,5P_3)[15–18], suggesting that these might mediate Ca^{2+} mobilization from internal pools[17,18]. Such an action of Ins1,4,5P_3 has recently been inferred from studies with permeabilized pancreatic acinar cells[19] and

hepatocytes[20,21]. Here we show directly that Ins1,4,5P$_3$ rapidly releases Ca^{2+} from a microsomal fraction of rat insulinoma but not from mitochondria or secretory granules. Moreover, this response is transient and desensitizes the microsomes to subsequent Ins1,4,5P$_3$ additions. These results suggest that Ins1,4,5P$_3$ functions as a cellular messenger inducing early mobilization of Ca^{2+} from the endoplasmic reticulum.

The ambient free Ca^{2+} concentration, $[Ca^{2+}]$, of the incubation medium maintained by the microsomes was determined using a Ca^{2+}-specific minielectrode[22]. We determined the specific activities of the apparent net Ca^{2+} uptake and of marker enzymes in four fractions obtained after centrifugation of the resuspended microsomes on a sucrose density gradient. Ca^{2+} transport activity correlated strongly with the endoplasmic reticulum enzyme marker but not with the plasma membrane marker (Fig. 1). This demonstrates that the high Ca^{2+} transporting activity of the insulinoma microsomes is localized mainly in the endoplasmic reticulum. As the experiments shown in Fig. 1 required long preparation times and such a large number of insulinomas, all subsequent work was carried out on the original microsomal fraction.

Figure 2*a* shows that insulinoma microsomes lowered the ambient free Ca^{2+} from ~1.5 μM to ~0.1 μM. At this point a pulse addition of Ins1,4,5P$_3$ resulted within 20 s in an increase in medium free Ca^{2+} to ~0.25 μM, followed by a decrease, representing Ca^{2+} re-uptake. Interestingly, a second addition of Ins1,4,5P$_3$ produced only a very small increase in $[Ca^{2+}]$, indicating that the increase in $[Ca^{2+}]$ after the first Ins1,4,5P$_3$ addition was not due to Ca^{2+} contamination of the compound. When a smaller, first pulse addition of Ins1,4,5P$_3$ was made, producing only a modest transient increase in medium $[Ca^{2+}]$, a second addition of Ins1,4,5P$_3$, even at a maximal concentration, elicited only a very small Ca^{2+} response (Fig. 2*b*). Similar observations were made whether the second addition occurred immediately after the first one or 40 min later. The reason for this phenomenon is unknown; it could conceivably reflect the process of densensitization, which is a feature of Ca^{2+}-mobilizing agonists[6]. Alternatively, during the first stimulation, an Ins1,4,5P$_3$-sensitive vesicle population released Ca^{2+} which was sequestered by a separate population insensitive to the action of Ins1,4,5P$_3$. A third possibility is that it was caused by a rapid degradation of Ins1,4,5P$_3$ and/or the appearance of degradation products of Ins1,4,5P$_3$. Experiments were done to explore this latter hypothesis. When microsomes were incubated in the presence of 9 μM Ins1,4,5 [4,5-^{32}P]P$_3$ (prepared and analysed as described previously[16,23]), 85 ± 7% (n = 3) of the label remained after 10 min, indicating that degradation was too slow to account for desensitization. Furthermore, the degradation products of Ins1,4,5P$_3$, that is, Ins1,4P$_2$, Ins1P and *myo*-inositol, all at a concentration of 10 μM, did not desensitize the microsomes to a subsequent Ins1,4,5P$_3$ addition.

The half-maximal effective concentration of Ins1,4,5P$_3$ was 3 μM and the maximal concentration was ~10 μM (Fig. 3). These values are somewhat higher than those reported for permeabilized pancreatic[19] and liver cells[20,21]. The ability to mobilize Ca^{2+} seems to be specific for the trisphosphate derivative because there was no release of Ca^{2+} on addition of Ins1,4P$_2$ (prepared as described previously[19,23]), Ins1P or *myo*-inositol, all tested at 10 μM (data not shown).

To test the organelle specificity of the response, insulinoma mitochondria were incubated in conditions similar to the microsome. The mitochondria buffered the ambient $[Ca^{2+}]$ to ~0.9 μM[22]. Ins1,4,5P$_3$ did not induce Ca^{2+} release nor did it change the extramitochondrial Ca^{2+} steady state. Ins1,4,5P$_3$ was not tested at a very low Ca^{2+} concentration (0.1 μM) as this causes Ca^{2+} depletion of the mitochondria. Secretory granules were unable either to lower the medium $[Ca^{2+}]$ or to take up a pulse addition of Ca^{2+}. Furthermore, of the large amount of Ca^{2+} that the secretory granules were found to contain on isolation, none was released by Ins1,4,5P$_3$. Thus, neither insulinoma mitochondria nor secretory granules appear to be sensitive to Ins1,4,5P$_3$.

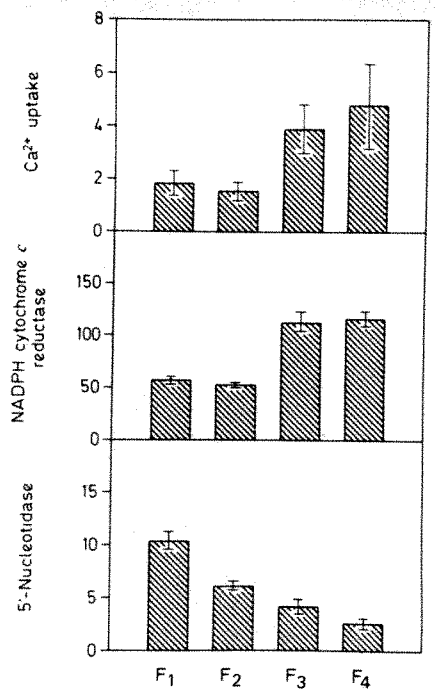


Fig. 1 Specific activities of MgATP-promoted Ca^{2+} uptake and of marker enzymes in four fractions obtained after centrifugation of the resuspended microsomal fraction on a discontinuous sucrose density gradient. Ca^{2+} uptake (nmol of Ca^{2+} per min mg protein) in the four fractions was measured with the same amount of material (0.2 mg protein ml^{-1}), as in Fig. 2 legend. The amount (nmol) of Ca^{2+} taken up by the four fractions was calculated from the reduction of the ambient free Ca^{2+} concentration on addition of each separate fraction (see also Fig. 2). For NADPH cytochrome *c* reductase, units are nmol of cytochrome *c* reduced per min mg protein; for 5′-nucleotidase, units are μmol adenosine formed per h mg protein. Values are the mean ± s.e. of four experiments.
Methods: Insulinomas were removed from rats[27,28], then dissected and homogenized as described previously[22]. Microsomes were obtained from the supernatant following three successive centrifugations: 1,500*g* for 5 min, 9,800*g* for 10 min, and 24,000*g* for 20 min. The resulting supernatant was centrifuged at 100,000*g* for 60 min to obtain a microsomal pellet. This fraction was enriched in NADPH cytochrome *c* reductase by 2.6-fold compared with the homogenate, 3.6-fold in 5′-nucleotidase, 0.01-fold in cytochrome *c* oxidase and 1.3-fold in immunoreactive insulin, the respective markers of endoplasmic reticulum, plasma membrane, mitochondria and secretory granules plus reticular structures. The microsomal pellet was gently washed (without disturbing the pellet) three times with medium containing 0.25 M sucrose, 5 mM HEPES *p*H 7.0 (sucrose medium) and resuspended in 0.1 ml of the sucrose medium, kept on ice and used immediately for Ca^{2+} transport experiments. In a separate series of experiments, resuspended microsomes were layered onto the surface of a discontinuous sucrose density gradient (1.5 ml of the densities 1.10, 1.14, 1.16, 1.20) and centrifuged at 150,000*g* for 90 min. Four discrete bands observed at the density interfaces were collected for protein, Ca^{2+} flux and enzyme marker determinations. These fractions were numbered 1–4 in order of increasing density (F_1–F_4). For the isolation of insulin secretory granules, the postnuclear supernatant was centrifuged at 23,700*g* for 15 min. The resultant pellet was resuspended, layered and centrifuged on a 30% Percoll gradient as described previously[22]. As the bottom of the gradient (see ref. 22) contained mainly insulin secretory granules, this fraction alone was collected. The final secretory granule pellet was obtained after three successive washings in sucrose medium at 23,700*g* for 15 min. The secretory granule fraction was enriched compared with the homogenate 11.2-fold in insulin, 1.1-fold in cytochrome *c* oxidase and 0.3-fold in NADPH cytochrome *c* reductase. Cytochrome *c* oxidase was assayed according to ref. 29; NADPH cytochrome *c* reductase was measured as described previously[30]; and 5′-nucleotidase was measured by the technique of Avruch and Wallach[31]. Immunoreactive insulin was assayed according to a method described previously, using rat insulin as standard[32]. Proteins were determined by the Coomassie blue method using a commercial kit (Bio-Rad).

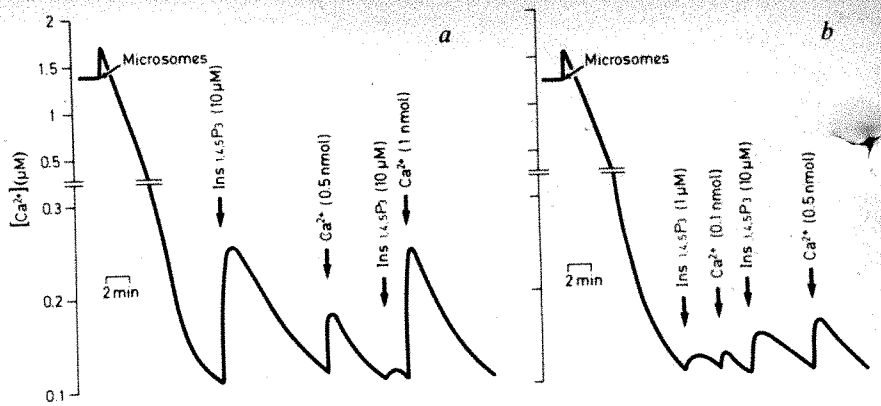


Fig. 2 Extramicrosomal ambient free Ca^{2+} concentration maintained by insulinoma microsomes: effect of Ins1,4,5P_3. Microsomes were incubated at 30 °C, *p*H 7.0 in 200 μl of a buffer containing 110 mM KCl, 2 mM KH_2PO_4, 25 mM HEPES, 1 mM $MgCl_2$, 1 mM MgATP, 3 mM creatine phosphate, 50 μg ml^{-1} creatine kinase, 0.2 μM antimycin and 0.5 mg ml^{-1} of bovine serum albumin, with continuous stirring. The medium [Ca^{2+}] was continuously recorded with a Ca^{2+}-selective minielectrode made and calibrated as described previously[22]. Where indicated, microsomes (0.75 mg protein ml^{-1}), Ins1,4,5P_3 or $CaCl_2$ (nmol per 200 μl) (in the amounts indicated) were added to the medium. Within 10 min, microsomes lowered the extramicrosomal ambient free Ca^{2+} concentration to ~0.1 μM; they were not easily saturated with Ca^{2+} as they could rapidly take up several sequential pulse additions (5 nmol per mg protein) of Ca^{2+}. The Ca^{2+} accumulated could be rapidly released by adding the Ca^{2+} ionophore A23187 (1 μg ml^{-1}) or by lowering the ATP present in the medium by the addition of glucose (5 mM) plus hexokinase (20 U ml^{-1}), indicating that Ca^{2+} is accumulated into vesicular elements in a MgATP-dependent manner (not shown). If microsomes were preincubated with the Ca^{2+} ionophore A23187 to empty the vesicles of Ca^{2+}, the addition of Ins1,4,5P_3 produced only a very small increase in medium [Ca^{2+}]. This amount of Ca^{2+} corresponded to a similar increase in [Ca^{2+}] when Ins1,4,5P_3 was added to the incubation buffer alone; it averaged ~5–10% of the effect shown in *a* and was accounted for in the subsequent calculation. Ins1,4,5P_3 was obtained by alkaline hydrolysis of ox brain phosphatidylinositol-4,5-bisphosphate, followed by preparative paper chromatography (R.F.I. and M.J.B., in preparation). The figure shows representative experiments which were repeated at least eight times.

Three lines of evidence suggest that Ins1,4,5P_3 induces Ca^{2+} release from the endoplasmic reticulum and not from plasma membrane vesicles contaminating the microsomal fraction. First, the insulinoma microsomal Ca^{2+} uptake correlated well with the endoplasmic reticulum enzyme marker, but not at all with the plasma membrane marker. Second, the amount of Ca^{2+} released by Ins1,4,5P_3 (10 μM) was 6.1 ± 0.2 nmol per mg protein (mean ± s.e. of six separate experiments). This is a considerable amount of Ca^{2+}—for comparison, most tissues, including pancreatic islets[1,2], have a total calcium content of ~20 nmol per mg protein. The Ca^{2+} is, therefore, unlikely to originate quantitatively from contaminating plasma membrane vesicles, in a tissue that is particularly rich in reticular structures such as insulinoma cells. Third, Ins1,4,5P_3 mobilizes Ca^{2+} in permeabilized pancreatic acinar and liver cells in which the plasma membrane should not function as a Ca^{2+}-storing organelle[19–21]. However, the possibility that Ins1,4,5P_3 could induce in intact cells an influx of external Ca^{2+} should not be precluded.

Agonist-induced rises in intracellular free [Ca^{2+}] have been universally associated with an enhancement of both phosphatidylinositol turnover and polyphosphoinositide hydrolysis[3–18]. More recent observations of a resultant, rapid, Ca^{2+}-independent generation of water-soluble inositol phosphates, have led to the hypothesis that these compounds could act as intracellular messengers mobilizing cellular Ca^{2+} stores[17,18]. This hypothesis is substantiated and extended by the present report. We have shown that Ins1,4,5P_3, at concentrations which are thought to occur in cells, can increase, within 20 s, the extramicrosomal concentration of Ca^{2+} from 0.1 μM to ~0.2–0.3 μM, corresponding respectively to basal or stimulated conditions in a variety of tissues[24–26]. The results of the present study strongly suggest a specific role for Ins1,4,5P_3 as a cellular messenger inducing Ca^{2+} release from the endoplasmic reticulum.

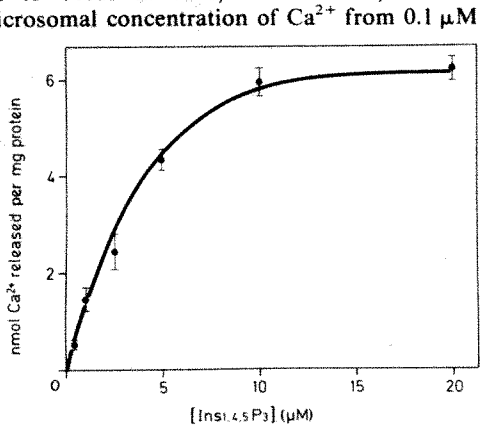


Fig. 3 Dose–response of Ins1,4,5P_3-induced Ca^{2+} release. Pulse additions of various known amounts of $CaCl_2$ (as shown in Fig. 2) were used to calibrate the Ca^{2+} rapidly released by Ins1,4,5P_3. Values are the mean ± s.e. of three separate experiments.

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Corrigendum

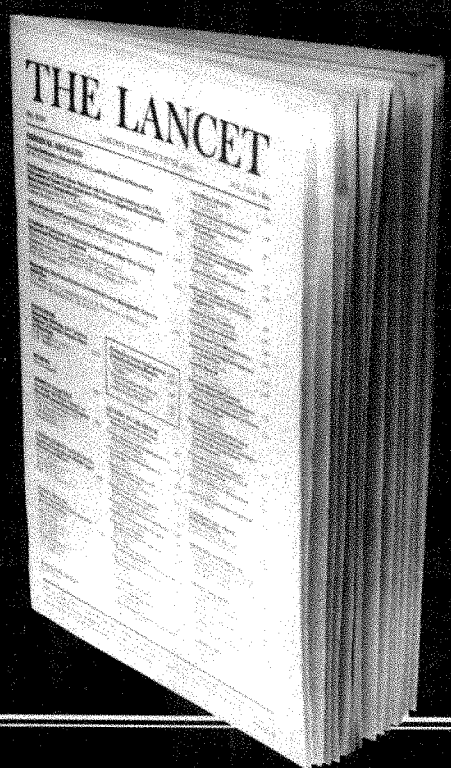
Therapeutic potential of monovalent monoclonal antibodies

S. P. Cobbold & H. Waldmann

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THE sentence on line 10 of the bold first paragraph should read: 'Starting from the original observation (by Glennie and Stevenson[3]) that rabbit antisera can . . . antigens'.

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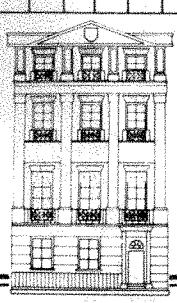
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WITHIN this work is an honest tale, plainly told. Therein lies both its strength and weakness. Its strength is that of the scientific tradition for unvarnished presentations of facts, letting them speak for themselves uncoloured by rhetoric, unstrained by advocacy, unheated by passion, unbiased by prejudice. For those raised in the scientific culture this is the natural, indeed the inevitable, mode of expression; the anvil on which every scientific deduction can be mounted and exposed to the hammer of critical analysis. Its weakness is that the scientific culture is a minority one. The great world which fashions public opinion — the world of hyperbole and shock-horror, of the self-inflated politician, bombastic trades union leader, committed journalist, trendy teleperson — resonates to a different sound. The anti-nuclear lobby know this full well. They are at home in this world and play its tunes as captivatingly as any Pied Piper.

Thus there is the extraordinary position today — nearly a third of a century after Calder Hall first brought nuclear electricity to Britain, a period in which the nuclear industry has gained a brilliant achievement in supplying cheap power, reliably and safely — that the nuclear industry is on the defensive, trying to justify itself to the general public in the only way it knows, with the quiet voice of scientific reason. For, as Sir Walter Marshall says in his preface to the three volumes, the aim was to prepare a work on nuclear power orientated towards increasing public knowledge and for the public interest. The project was conceived at a time when the United Kingdom Atomic Energy Authority, of which the then Dr Walter Marshall was Chairman, was approaching its twenty-fifth anniversary (in 1979). This event could have justified a little trumpet-blowing, but instead the decision was to review the state of nuclear science and technology in the classical scientific manner.

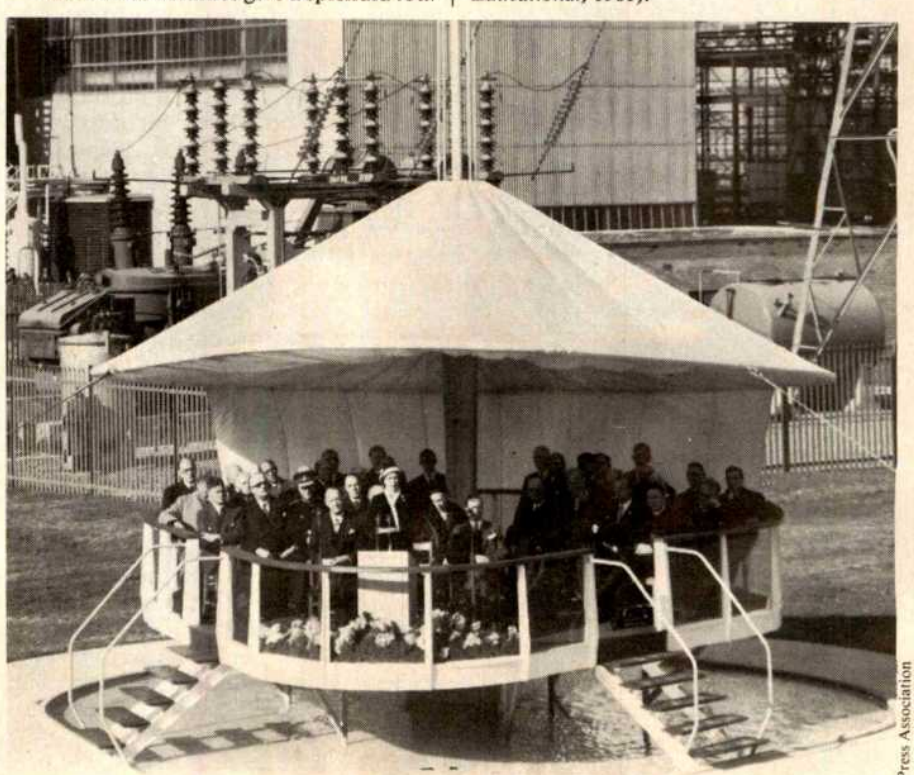
This is done in 24 chapters, each by different authors who come almost without exception from within the UKAEA. Such a division of the labours is probably the only way to deal comprehensively and authoritatively with such a huge and wide-ranging subject. It has of course the usual advantages and disadvantages. On the merit side each individual topic is given a chapter and author(s) to itself, and so enjoys a reasonably self-contained exposition which can be read separately from the rest of the contents; against this, there is inevitably some repetition of underlying points that are common to more than one chapter.

The eight chapters in Vol.1 deal with nuclear reactors. After two introductory contributions, which outline the underlying physics and assess the current status of thermal and fast reactors — and go surprisingly far into the mathematics of neutron physics, in a work intended for the public — the various main reactor types are described, namely gas-cooled, light water, fast and heavy water. Two final chapters deal with novel reactor concepts, for example fluid-fuel reactors, and briefly with prospects for fusion power. Volume 2 covers the various aspects of the fuel cycle — occurrence, supply and enrichment of uranium, fuel design and fabrication, reprocessing, waste management, decommissioning, thorium fuel cycles — in similar style. Volume 3, which is perhaps of greatest interest to the general public, deals with various aspects of nuclear radiation — biological, detection, environmental, risk assessment, medical radioisotopes. Radioactive dating is also covered, although surprisingly there is no chapter on the use of gamma radiation in industry or on non-medical applications of radioisotopes.

These three volumes give a splendid *tour d'horizon* of the science and technology of nuclear power. Not surprisingly, given their provenance, the expositions centre round British experience and points of view. Nevertheless much of the treatment is sufficiently general as to be of interest to readers in other nations, especially in countries having light-water reactors, for these are dealt with almost as fully as gas-cooled reactors. The treatment of fast reactors is also detailed. Perhaps, if only in fairness to the outstanding Canadian effort, there might have been a little more about the excellent CANDU reactors.

Finally, however, the questions which began this review raise themselves once more. I am sure that many people within the nuclear industry, as well as some scientists and engineers outside it, will read these books with both pleasure and profit. But will the general public and all those publicity-seeking pundits who pronounce so readily and volubly on all matters nuclear? I doubt it. The first group probably does not have the time to digest all the facts so fully gathered together here. The second bask in their unfettered imaginations and have no need of facts. The best response I can make to this work is to thank the authors for a noble effort, which has produced a fine work of science, and to tell them that something entirely different is needed to win the hearts and minds of the great majority in the non-scientific culture. □

Sir Alan Cottrell is Master of Jesus College, Cambridge. Among other books he is author of How Safe is Nuclear Energy? (*Heinemann Educational, 1981*).



Press Association

Day of confidence — Wednesday 17 October, 1956, and the Queen opens Calder Hall, the first full-scale nuclear power station.

Hormone balance

S.J. Simpson

Endocrinology of Insects. Invertebrate Endocrinology, Vol.1.
Edited by Roger G.H. Downer and Hans Laufer.
Alan R. Liss/Wiley: 1984. Pp.707. $199.80, £111.

ONE of the most daunting tasks in biology would be to prepare a volume entitled *Endocrinology of Insects*. So daunting, in fact, that until now no one has attempted to do more than review selected aspects of what is an enormous, complex and rapidly expanding field. Downer and Laufer have approached the problem of producing a detailed, scholarly review of the subject in exactly the right, and probably the only way: they have asked specialists to cover defined ground.

The resulting volume deals with the nature of endocrine glands and neurosecretory cells; the chemistry and biosynthesis of hormones, and their interaction with target cells; intracellular second-messengers; the physiological role of hormones; myotropic and neurotropic substances; pheromones; the relevance of insect hormones in plants; and the usefulness of hormones in insect control. Inevitably there are omissions, and problems of redundancy and lack of continuity, but the volume as a whole is a success. The differing interpretations and standpoints expressed by the contributors do, as the editors claim, provide depth and balance to the work.

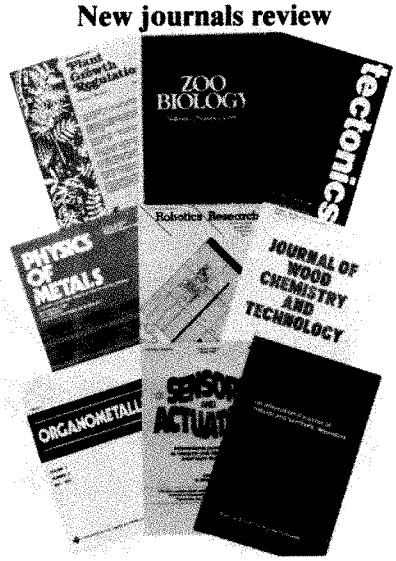
Endocrinology of Insects is not easy to read; not, in most cases, because the writing is unclear, but because the authors have done justice to their topics. This approach has often precluded the drawing of generalizations or syntheses. Even the introductory overviews for such sections as "Regulation of Metamorphosis", "Regulation of [Female] Reproduction" (unfortunately male reproduction is not represented) and "Metabolic Homeostasis" are, by necessity, unable to do more than draw together information in a superficial sense. In most cases, not enough is known to generalize, and what *is* known is often conflicting — different species use the same hormone for different purposes; different strains of the same species, and even a single strain under different rearing regimes, give conflicting results; it is difficult to distinguish real effects from those stemming from dissection and bioassay techniques; and there is a danger that phenomena are attributed to a particular hormone when in fact the hormone is acting at an earlier stage in a causal sequence involving other endocrine and neural factors. More aspects of insect endocrinology will, perhaps, soon begin to gel, but the cautious approach of many of the contributors is generally justified.

An unusual aspect of the book is the coverage of areas about which virtually nothing is known. Thus the section on metabolic homeostasis includes three detailed chapters on excretion, on lipid metabolism and on carbohydrate metabolism, along with a chapter on nitrogen metabolism in which the word hormone is almost absent. Such a contrast emphasizes those areas which are poorly understood and puts the better known subjects in perspective. The quest for completeness has its drawbacks, however. For example, neurotransmitters, neuromodulators and pheromones differ from hormones in degree rather than kind and the editors have chosen to represent them but briefly — in the case of pheromones, too briefly to be of much use.

I would have liked more thorough cross-referencing between chapters, but that apart my main worry is that the book is two years out of date. Nonetheless, *Endocrinology of Insects* is a very important work. An understanding of hormonal mechanisms is essential to anyone who studies insect behaviour or physiology, and this book makes a difficult subject accessible to us all. □

S.J. Simpson is Demonstrator in the Department of Zoology, University of Oxford.

New journals review



On 27 September *Nature* will publish the fourth annual review supplement devoted to science journals.

Criteria for inclusion of a journal in the 1984 issue are that:

(i) the first number appeared, or the journal was retitled, *between June 1982 and May 1983* (the second cut-off date allows at least three issues of a journal to have been published, the minimum number on which a reasonable judgement can be based);

(ii) it is published at least three times a year;

(iii) the main language used is English.

Publishers and learned societies are invited to send *four different issues of each suitable periodical, including the first and most recent numbers* (if from outside the United Kingdom, by air mail) to: The Review Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF, England. Subscription details, if possible for both 1984 and 1985, should be included.

Screening methods of recombination

E.J. Wood

The Techniques in Genetic Engineering Video Library. TGE 1: Nucleic Acids Techniques — An Overview.
By Stephen D.M. Brown.
IRL Press: 1984. Formats available: PAL in VHS and Betamax, £80, $160. U-matic copies £7.50, $15 extra. Supplementary charge for NTSC and SECAM standards conversions.

THIS 25-minute videotape is the first of a series of eight* which will deal with the techniques of genetic engineering. Viewed in isolation from the rest of the programmes (which will appear later this year), it gives a foretaste of the high quality of production of the series, but tells us less about what can be expected of the content and level of the subsequent tapes. Nor is it yet clear how easily a student could proceed from instruction of this type to actual work in the laboratory.

The programme starts with a graphic display of a computer-generated DNA double helix, the introductory section then featuring a series of stills during which the voice-over talks about the impact of genetic engineering and its potential and limitations. A brief interview with Sir Gordon Wolstenholme gives an historical view of developments since Watson and Crick, and stresses that no one really saw any practical uses for the techniques of genetic manipulation until seven or eight years ago.

Following on, the fundamental techniques underlying recombinant DNA technology are explained, namely the use of restriction enzymes, of sticky ends and of ligases. The use of restriction enzymes is well illustrated with graphics, although I wonder if it is really necessary at this level to speak about the different classes of restriction enzyme. Analysis by restriction mapping is demonstrated, and from this a student would readily get a good feel for how slabs of agar are used in electrophoresis, blotting and so on. On the whole this section is evenly paced, well-explained and clearly photographed.

The next section deals with the cloning of DNA into a phage in order ". . . to look at a single gene". Graphics show the ligation of a section of "human" DNA into phage DNA, reinforcing what had been explained in the previous section. However at this stage the graphics seem to get out of phase with the voice-over: while the latter talks about plaques in a lawn of bacteria, the graphics show thousands of virus particles.

* Titles to follow are *Gene Analysis and Southern Blotting; DNA Sequencing with M13; Gene Libraries; Expression of Cloned Genes; Oligonucleotides — Their Use in Gene Cloning; Site-Specific Mutagenicity;* and *Microdissection and Microcloning.*

This section of the programme is the most uneven — speeding through a number of techniques (clone selection, blotting, hybridization, DNA sequencing) would surely leave the student wondering what the techniques are for and not really knowing how to carry them out. No doubt such details come in a later programme, but here the producers should have resisted the temptation to pack in so much detail.

The pace then slows down, as *c*DNA cloning is dealt with briefly but adequately, but speeds up again as we launch into monoclonal antibodies and start injecting things into *Xenopus* oocytes. Well, it *is* an overview; but again I would have been happier had the desire to mention almost every technique been stifled.

The final section largely uses stills with voice-over to try to answer the question: "Why is industry so interested in all this?". Topics include improving the quality of plant proteins, mineral extraction, "improved" bacteria for energy production from plant waste and for degrading pollutants, as well as interferon and insulin production. Much credit is given to genetic engineering (at the molecular level) for doing, or potentially doing, all of these things; but it is, I think, churlish, if not inaccurate, to fail to mention that for centuries plant and animal breeders have been messing around with the genetic constitutions of species in order to "improve" them.

Also included in the final section is a description of how recombinant DNA technology may, and indeed is, being used in the diagnosis of inherited disease. How this is done is very clearly shown including a description of the analysis of DNA taken from chorionic villi using a DNA probe for prenatal diagnosis of a genetic defect.

Overall the programme gives a good indication for what can be done with recombinant DNA. The photography of the techniques is of a high standard, illustrating the advantage of film or videotape over "cold print" for demonstrating methodology, and most of the graphics are clear and straightforward. The speech, too, is clear and should easily be understood by those whose first language is not English. My main criticism is the unevenness of pace in some parts of the programme, but I would expect this to be less of a problem in subsequent tapes dealing with individual techniques in detail.

A little booklet accompanies the videotape and contains many of the graphics in printed form. These diagrams will be useful for students when revising the details of the techniques. However, while the booklet gives a warning that some of the reagents used are hazardous, neither tape nor booklet says that you should get the permission of a genetic manipulation committee before proceeding. Perhaps this comes later. □

E.J. Wood is a Senior Lecturer in the Department of Biochemistry, University of Leeds.

On ice and climate

Claus U. Hammer

The Climatic Record in Polar Ice Sheets.
Edited by Gordon de Q. Robin.
Cambridge University Press: 1983.
Pp.212. £32.50, $59.50.

GLACIERS move slowly. So, apparently, does the publication of monographs on polar ice sheets. In *The Climatic Record in Polar Ice Sheets*, an offspring of a workshop held in 1973, the subject is the information to be obtained on past climate from ice sheets, through the past 100,000–150,000 years with a few excursions further back into the Quaternary. The huge polar ice sheets are indeed slowly moving, frozen libraries of past atmospheric conditions, but the information they contain is written in a detailed, complex language. How far have we come in the translation of that language?

The aim of the workshop was to investigate the validity of data obtained from ice sheets, whether in the form of isotopic composition of ice cores or via measured temperature profiles along the corresponding boreholes. Such studies are complicated by the flow history of the ice, and changes in the isotopic composition of the ice can only in part be interpreted as changes in temperature. The book offers a great deal of detailed information on these problems, and a central much-needed contribution (Chapter 5) is devoted to ice flow and heat transport within the ice sheet.

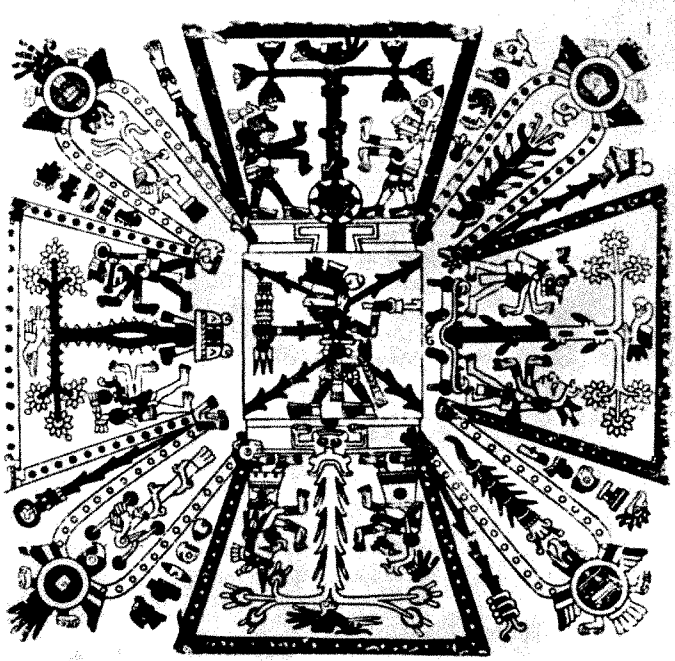
The general reader may have difficulties getting through this account, though glaciologists will feel more at home. The model calculations presented are exemplified by ice core case-studies, but I suspect that current models and input parameters are not realistic enough to give safe answers; a lot more glacio-meteorological data are needed before climate may be accurately inferred from ice cores.

In other chapters the reader is given a guided tour of the ice library, but I cannot help feeling a little lost: lacking is an introductory chapter to place the enterprise within the general context of the Quaternary. Attempts to this end have been made in a chapter on ocean cores, but a broader discussion of time scales and variability of ocean core data would have been helpful. Nonetheless, the data presented in the book add much to our understanding of salient problems in this area. For example, the total gas content in air bubbles in the ice can indicate old surface elevations of the ice sheets, and surface studies of snow accumulations, temperatures and so on are clearly of importance.

The book's emphasis on the relationship between isotopes and borehole temperature profiles is more a reflection of its origin at a workshop in 1973 than of new developments. While contributions have been updated by the introduction of more recent results, they do not entirely conceal the fact that ten years have passed since the book was conceived. That being said, some of the most important findings in ice-sheet–climatic research since the 1960s *are* presented here, among them a number bearing upon still-controversial subjects.

This collection of ice-core data with its discussion of analytical methods is a must for climatic researchers. But to interpret much of the information found in the book such readers may require additional information from the glaciological literature. □

Claus U. Hammer is at the Geophysical Isotope Laboratory, University of Copenhagen.



The cosmological plan of the Aztec universe, laid out horizontally. The illustration shows the nine Lords and two hundred and sixty days of the Sacred Calendar assigned to the four cardinal directions, the centre of the universe, and sacred birds and trees. The illustration, reproduced from the *Codex Fejervary-Mayer I*, is taken from Brian M. Fagan's *The Aztecs*, an illustrated account of early Mexican life and culture. The book is published by W.H. Freeman, price hbk $27.95, £26.95; pbk $14.95, £13.95.

Evolution and us

John Beatty

Philosophy, Evolution and Human Nature.
By Florian von Schilcher and Neil Tennant.
Routledge & Kegan Paul: 1984. Pp.283. £16.95, $29.95.

THIS is a thought-provoking book — the result of a brainstorming collaboration between a philosopher (Tennant) and a behavioural geneticist and evolutionist (von Schilcher). The authors are mainly concerned with the ramifications of adaptationist accounts of human nature. They succeed in demonstrating what one might call (only partly tongue-in-cheek) the "resourcefulness" of the adaptationist perspective, and also do *pretty* well in countering current methodological and epistemological critiques of adaptationist theorizing. But their apparent reasons for making adaptation the centre of attention of a scientific study of human nature include some contentious views about evolutionary biology.

Following a long introduction to the structure and conceptual foundations of evolutionary theory (which is sound, but which could have been improved by incorporating at least *some* of the recent work in philosophy of biology), the authors turn their evolutionary lens upon their own kind — upon human values, cognition and language. As for values, they warn against the "naturalistic fallacy" of those who pretend to derive normative conclusions from purely evolutionary premises, but warn also against the "anti-naturalism" of those who believe that the facts of evolution are entirely irrelevant to ethics. As for cognition, they concentrate upon the "evolutionary exegesis of Kant": to what extent, they ask, are the categories of cognition grounded in neurological structures that are, in turn, the products of evolution by natural selection. They acknowledge, in this regard, some of the problems that result from "spiking" the brain with "an overly rich mixture of cognitive spirits". And, as for language, they are interested in the extent to which the individual development of grammatical skills "recapitulates" the phylogenetic history of grammatical innovations.

Adaptationist accounts of human nature are certainly worth entertaining. But I question a number of Tennant and von Schilcher's underlying reasons for pursuing such an approach, some of which seem to me to be based on serious misrepresentations of the structure and domain of evolutionary biology. First, they misconstrue evolutionary theory as being underpinned by nothing more than principles of natural selection, and evolutionary biology as being nothing more than the study of adaptations. Then they make unjustifiably bold claims about the domain of evolutionary biology — that is, about how much we can expect to explain in terms of evolution by natural selection.

For instance, among the "components" of the modern synthetic theory of evolution they include "The postulation of natural selection working on random genetic mutations as the one and only mechanism of evolutionary change" (p.97). And yet most versions of the Hardy–Weinberg law (the central principle of the synthetic theory) cite natural selection, mutation, migration, random drift and non-random mating as possible means of evolutionary change.

Even when von Schilcher and Tennant acknowledge alternative mechanisms of evolution, random drift for example, they belittle the search for such phenomena. For instance, they claim that

> the pan-selectionist point of view is the only possible working hypothesis. This is simply because someone not searching for a function [i.e. adaptation] will never find one, even if one should exist [p.63].

But surely it would be just as unlikely for someone to find an instance of random drift if they were not looking for it. In other words, there may be a good reason for saying that the pan-selectionist point of view is the only possible working hypothesis, but von Schilcher and Tennant have not provided it.

Finally, consider the authors' bold *a priori* extension of biological evolutionary theory to cultural evolution:

> *A priori* biological evolution is going to produce culturally adapted individuals when an important part of the environment is cultural; and genotypes placed in such environments will produce phenotypes with important cultural traits [p.113].

And they add:

> to discern a causal interaction between genes and culture is not to make a category mistake; indeed, its denial is a logical contradiction once culture is reckoned to the environment, cultural traits are reckoned to the phenotype, and the core conditional of evolutionary theory is understood just as before [p.113].

I believe that the authors have seriously overestimated the power of pure reflection here, and have tried unsuccessfully to turn an empirical, scientific issue into a philosophical one.

In one sense fortunately, and in another sense unfortunately, the authors' stimulating account of the ramifications of an adaptationist approach to human nature does not entirely depend upon their having established the all-importance of evolution by natural selection. It is fortunate in that their discussions, and many of their arguments, are still well worth consideration. It is, however, unfortunate in that the needless mistakes are distracting indeed. □

John Beatty is an Associate Professor of Philosophy at Arizona State University, Tempe, Arizona.

Paper crust

Princeton University Press have re-issued in paperback *The History of the Earth's Crust*, edited by Robert A. Phinney, the proceedings of a conference held in 1966 which proved to be a landmark in the earth sciences. The book was first published in 1968. Price of paperback is $11.50, £10.

Aspects of genius

R.S. Woolhouse

Leibniz. By G. MacDonald Ross.
Oxford University Press: 1984. Pp.121. Hbk £7.95, $12.95; pbk £1.95.

LEIBNIZ (1646–1716) was a multifaceted genius. He was archivist, diplomat, engineer, inventor, librarian, poet and politician. And his vast output of writing covered alchemy, anthropology, dynamics, economics, geology, history, jurisprudence, linguistics, logic, mathematics, numismatics, philosophy and religion.

The introduction to George MacDonald Ross's clear and very readable contribution to the *Past Masters* series prides itself on recognizing this fact. It leads us to expect the novelty of a discussion of all aspects of Leibniz's work and thought. But, after the initial notice taken of them in the valuable account of his life, most are ignored. As usual it is Leibniz's philosophy and parts of his mathematics that really receive the attention.

As even this is a lot to cover in a limited space it need be no surprise if this short book is not without flaws. Though Leibniz indeed says otherwise, it is not true of the historical Descartes that he squared human behaviour with his law of motion-conservation by making the soul change only the *direction* of motion of brain particles, not the *amount*. And though Leibniz did question how mind could possibly act on body, this was a background metaphysical worry. It was not a particular response to an attempt to make that action consistent with believed empirical laws. His specific objection to the manoeuvre he attributed to Descartes was that though it saved Descartes's (in fact false) law of motion-conservation, it did not save the (true, but unknown to Descartes) law of momentum-conservation.

Yet MacDonald Ross's achievement in introducing and summarizing Leibniz is considerable. Inevitably he compresses and omits. But he does not generally resort to superficialities, or rely on vague and general gestures. As a whole the book is fresh, stimulating and very informative. □

R.S. Woolhouse is Senior Lecturer in Philosophy at the University of York, and editor of Leibniz: Metaphysics and Philosophy of Science *(Oxford University Press, 1981).*

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FIELD: Virology/Epidemiology.

GENERAL: CSIRO's Division of Wildlife and Rangelands Research undertakes research into the general principles underlying the control of animal and plant populations and the application of this knowledge to ecosystem mangement and conservation. One of the research teams at the Division's laboratories at Canberra has conducted long-term fundamental field and laboratory studies of the ecology, physiology and behaviour of the European rabbit over the past 25 years, and of its response to and interaction with myxoma virus.

Recent field experiments have shown that myxomatosis continues to control rabbits in agricultural regions. In the semi-arid inland of Australia however, rabbit numbers still fluctuate greatly over wide areas, and are largely unchecked by some outbreaks of myxomatosis which are spasmodic. Major questions are how and where does the myxoma virus survive between epidemics, what are the reasons for failure of some outbreaks to control rabbits, what threshold level of infection in the population facilities spread by vectors, and what limits the rate of spread of the virus on both sides of that threshold.

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(NW589)A

Yale University

The European Molecular Biology Laboratory, a research institute situated in Heidelberg, West Germany, invites applicants for the following vacancy.

TECHNICAL ASSISTANT

The successful applicant will work in a group concerned with the cloning of mammalian genes. There will be a strong emphasis on DMA technology and microbiological techniques.

Appropriate training in the above mentioned fields is essential. It would also be desirable if the candidate had previously been introduced to working with computers and physical methods. A working knowledge of English or German is required; knowledge of a second language (English, French, German) is desirable.

An above average salary will be offered to the successful candidate. Certain allowances are payable in addition, depending on personal circumstances. An initial contract of 3 years' duration which can be renewed, will be offered.

Please write briefly for an application form, quoting reference no 84/ 26 to:

EMBL Personnel Section, Postfach 10.2209, D-6900 Heidelberg.

(W1024)A

HEWLETT PACKARD

Managers for advanced research programmes

In all areas of Computer Science

Up to £35,000+substantial benefits

We are searching for a number of key scientific trail blazers to direct major research projects at the new Hewlett-Packard Research Centre, Bristol.

The Hewlett-Packard commitment to technical excellence is acknowledged in world markets. It has achieved a prominent position through the calibre of its research effort in Palo Alto. In 1983, the company spent $493m on R&D, representing 10.5% of sales revenue.

A further, and highly significant, example of Hewlett-Packard's commitment to the future, is the establishment of a new Research Centre in Bristol. The rationale for this development, centres on the recognition that in Europe, there are areas of particular expertise, which, if brought to bear, will significantly contribute to Hewlett-Packard's long term corporate product objectives.

Our immediate task is to recruit a number of managers, to drive specific project teams in the following areas:

- ★ Communication Networks/Data Coding and Encryption.
- ★ Artificial Intelligence/Expert Systems.
- ★ Software Engineering — developing tools and methodologies.
- ★ Graphics.

For these critical roles we are looking for people of very high calibre. They will be acknowledged experts in their field and have the management skills to lead talented teams creating some of the future generation of Hewlett-Packard products. Clearly we will be asking for high academic achievement, together with a record of creativity and implementation of design concepts, which has led to the successful introduction of products into the market place.

The managers appointed will establish close working relationships with their opposite numbers in Palo Alto, Universities and with Hewlett-Packard operating companies in Europe. Communications between Bristol and Palo Alto will be close through regular visits and the introduction of a teleconferencing facility. The Bristol Laboratory will conduct both unique and complementary research to its counterpart in Palo Alto. It will grow to a total of some 300 people by 1987.

If you would like to explore these opportunities further, send your curriculum vitae in confidence to Geoffrey King, Managing Director of Cambridge Recruitment Consultants, who is advising Hewlett-Packard on these appointments, which are open to both men and women.

Cambridge Recruitment Consultants

1a Rose Crescent, Cambridge CB2 3LL. Telephone: 0223 311316.

(2301)A

Selly Oak Hospital

PRINCIPAL BIOCHEMIST

Applications are invited from graduate scientists with a Clinical Chemistry and/or Rheumatological Biochemistry background and with proven research ability for a Principal Biochemist post as Head of the newly established routine and research Rheumatological Biochemistry Section in the Biochemistry Department at Selly Oak Hospital. The person appointed would work in close co-operation with the Birmingham University Department of Rheumatology and it is anticipated he/she would receive an appropriate honorary university appointment.

Full particulars, and application forms, from Mr. P. Scott, Head of Biochemistry Department, Selly Oak Hospital, Birmingham B29 6JD. NHS Whitely Council conditions of service apply. Applications, including CV and names of 2 referees, to Mr. P. Scott by 19 June, 1984. (2248)A

SOUTH BIRMINGHAM Health Authority

PRODUCT MANAGER

DNA SEQUENCING

BIO-RAD LABORATORIES, a leading biotechnology company located in the San Francisco Bay Area, has as a result of our continuing growth an immediate opportunity for an experienced professional to work as a part of our marketing team in the Research Products Group. We are seeking a highly motivated individual with a commitment to excellence who is ready for the next challenging career step.

This position requires DNA sequencing experience and familiarity with computer analysis techniques. The successful candidate will be results oriented, a good communicator, have strong marketing potential, and be willing to travel. Position includes but does not emphasize lab work, along with multidiscipline project management responsibilities.

Bio-Rad offers a competitive compensation package including comprehensive benefits and relocation assistance. If you would like to be considered for this position, please submit your resume, including salary history, to Human Resources Dept. #167, BIO-RAD LABORATORIES, 2200 Wright Avenue, Richmond, CA 94804. An equal opportunity employer.

(NW591)A

BIO-RAD *Bio-Rad Laboratories*

THE UNIVERSITY OF SYDNEY

LECTURESHIP IN INORGANIC CHEMISTRY

School of Chemistry

(Reference No 20/03)

The Lecturer will participate in teaching at the elementary, advanced undergraduate, and postgraduate levels. The department has active research groups in organometallic chemistry, bio-inorganic chemistry, analytical and environmental chemistry, crystal structure analysis, vibrational spectroscopy, coordination dynamics and molecular electro-optics. Preference may be given to applicants whose research specialisation is in applications of spectroscopic methods to biological inorganic chemistry.

The appointment will commence in or after September 1984.

Salary range: $A24,353-$31,994 per annum (under review).

Appointments to lectureships are usually probationary for three years and the University reserves the right not to proceed with any appointment for financial or other reasons. The costs of travel to Sydney by the appointee may be paid by the University.

Applications, quoting reference no. and including curriculum vitae, lists of publications and names of three referees, to the Registrar, University of Sydney, NSW 2006, Australia, by 20 July 1984.

For further information contact Professor H C Freeman in the University. (Telex AA20056).

Further general information also available from Association of Commonwealth University (Appts), 36 Gordon Square, London WC1H OPF.

(W1033)A

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT IN MOLECULAR BIOLOGY

Applications are invited for a postdoctoral position to identify sequences in the β gene of E.coli RNA polymerases necessary for enzyme function. The work will involve the use of genetic and biochemical techniques, including DNA sequencing, to locate the sites of substitutions known to perturb RNA polymerase activity in vivo. It will lead to the generation of a structure-function map.

This Grade 1A MRC-supported position, tenable for three years, has a starting salary of £7,190 per annum including USS benefits.

Applicants should send a curriculum vitae and the names of two referees to Dr Robert E Glass, Department of Biochemistry, Queen's Medical Centre, Nottingham, NG7 2UH (Telephone 0602-700111 extension 3395) from whom further information may be obtained. (2258)A

KINGSTON POLYTECHNIC

Faculty of Science

DEPARTMENT OF GEOLOGY

LECTURERII/SENIOR LECTURER IN GEOLOGY

(Ref: GG2)

2 posts:

Petrology/Geochemistry

Sedimentology)

Applications are invited for two posts which are available from 1st September. Applicants should have a high research potential and a strong interest in teaching. Preference will be given to candidates with experience in petrology/geochemistry and sedimentology, particularly of carbonate rocks. The successful candidates will be expected to take a lead in the research work of the Department and a willingness to make industrial contacts with a view to developing consultancy will be encouraged.

DEPARTMENT OF MATHEMATICS

LECTURER II/SENIOR LECTURER IN STATISTICS

(Ref: MA1)

Applications are invited for the above post, available from 1st September. Preference will be given to candidates with a postgraduate qualification in Statistics and/or industrial or consultancy experience. Recent involvement in Statistical Modelling or Optimisation would be an additional advantage.

Salary range Lecturer II/Senior Lecturer: £7860-£14,088 (under review) inclusive of London allowance. Please quote reference when applying.

Further details and application forms (to be returned by 29th June) from Personnel Officer, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. Tel 01-549 1366 ext 287. (2283)A

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF BIOCHEMISTRY

WELLCOME LECTURER

Applications are invited for the above post made available to the Department of Biochemistry by the Wellcome Trust. The appointment will be for three years in the first instance renewable for a further two years subject to satisfactory review. Salary will be in the range £7,190-£14,125 pa (under review).

Candidates, who should be under the age of 35, should have relevant experience in nucleic acid biochemistry. it is envisaged that the successful candidate will join a nucleus of staff interested in aspects of toxicology.

Applications (3 copies), the names of at least two referees together with a 500-word summary of proposed research should be forwarded to the Vice-Principal (Administration) and Registrar, University College, PO Box 78, Cardiff CF1 1XL, from whom further particulars may be obtained. Closing date 31st August, 1984. Ref: 2789 (2249)A

THE ROYAL VETERINARY COLLEGE
University of London

Division of Preclinical Studies

DEPARTMENT OF PHYSIOLOGY, BIOCHEMISTRY AND PHARMACOLOGY

TEMPORARY LECTURER IN VETERINARY PHARMACOLOGY

Applications are invited for a one-year temporary lectureship funded by the Wellcome Trust and available from 1 October 1984, or soon thereafter. Teaching duties will be on the preclinical veterinary pharmacology course. Applicants will be encouraged to pursue research work with Dr P M Headley on the actions of analgesic agents in vivo and in vitro.

Candidates should be postdoctoral and have experience of neurophysiological and neuropharmacological techniques. Ideally they will also have some experience of veterinary practice and/or teaching. Salary on the Lecturer scale up to £11,061 pa, including London Allowance. Salary will be determined by qualifications and experience.

Application form available from the Assistant Secretary (Personnel) The Royal Veterinary College, Royal College Street, London NW1 0TU (Tel: 01-387 2898 ext 230).

Prospective applicants should contact Dr P M Headley as soon as possible. Closing date 8 July 1984.
(2280)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF OCEANOGRAPHY

Applications are invited for the post of

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

Assistant in the Department of Oceanography.

This post is funded under the NERC Estuarine Processes Special Topic Programme for a three year period beginning 1 October, 1984. The project is for work with Dr M R Preston in collaboration with Dr R F C Mantoura (Institute for Marine Environmental Research, Plymouth) on comparative studies of some organic traces in estuarine particulates. Candidates should preferably have experience of GC and HPLC techniques and should be prepared to divide their time approximately equally between the Liverpool and Plymouth Laboratories. Informal enquiries may be made to Dr M R Preston, tel: 051-709 6022 ext 2699.

Initial salary will be £7,190 per annum.

Applications, together with the names of three referees, should be received not later than 25 June, 1984 by The Registrar, The University, PO Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/531/N.
(2231)A

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

DEPARTMENT OF MOLECULAR ENDOCRINOLOGY

Applications are invited from suitably qualified candidates for appointment as Lecturer within the Department of Molecular Endocrinology. The successful candidate will be primarily responsible for the day-to-day scientific and organisational management of a monoclonal antibody production unit forming part of the Supra-regional Assay Service (Hormones) Laboratory.

The post requires considerable experience and proven ability in hybridoma technology, the capacity to independently pursue effective methodological development in this field and an aptitude for teaching at a variety of undergraduate and post-graduate levels.

The successful candidate will also be encouraged to collaborate in studies on the development of the foetal central nervous system involving monoclonal antibody-based cell recognition techniques. Experience in this area, though advantageous, is not essential.

The salary will be within the Lecturer's scale and will be set at a point within the range commensurate with the responsibilities attachng to this appointment, and in accordance with age, qualifications and experience of the successful candidate.

Applications should be submitted to Professor Roger Ekins, Department of Molecular Endocrinology, The Middlesex Hospital Medical School, Mortimer Street, London W1N 8AA, together with the names of two referees, by 28 June, 1984.
(2292)A

BLOOMSBURY HEALTH Authority, Hospital for Tropical Diseases, London, England. Consultant Parasitologist. (Whole time/maximum part time). Applications are invited for the post of whole time/maximum part time Consultant Parasitologist. The successful candidate, who must be medically qualified, will be head of the Department of Parasitology providing a specialist diagnostic service. Close links exist with the London School of Hygiene and Tropical Medicine and it is anticipated that the postholder will have a fairly high academic commitment with appropriate honorary status. Those unable for personal reasons to work full time will be eligible to be considered for the post. Informal enquiries may be made by telephoning Dr M F R Waters, The Hospital for Tropical Diseases 01-387 4411. Application forms and job description available from the Medical Personnel Department, Bloomsbury Health Authority, 25 Grafton Way, London WC1E 6DB, or telephone 01-387 7643 (24 hour service) quoting reference PK/11. The closing date for receipt of applications is 24th August, 1984. Overseas candidates are invited to cable their intention of applying and send a curriculum vitae direct. An equal opportunities employer. (2238)A

CSIRO
AUSTRALIA

RESEARCH FELLOW — PLANT MOLECULAR BIOLOGY

$A25,342-$A37,274

DIVISION OF HORTICULTURAL RESEARCH
ADELAIDE SA

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Horticultural Research is a member of the Institute of Biological Resources.

FIELD: Nuclear/chloroplast interactions.

GENERAL: The aim of the Division's research is the improvement of woody perennial horticultural crops in Australia. These include a range of deciduous and evergreen species. Emphasis is placed upon the development of new techniques for the selection and breeding of improved plant type and on understanding the complex interaction between plant performance and the environment. The Division undertakes applied and basic research on a range of perennial horticultural plants (deciduous and evergreen) from both temperate and sub-tropical regions.

Intending applicants from UK and Europe may wish to make arrangements to talk to a member of the group who will be in Europe in July 1984.

LOCATION: The Division has laboratories at Adelaide, South Australia (Headquarters) and Merbein, Victoria. The appointee will be located at Adelaide.

DUTIES: As a member of a small team involved in studying the interaction of the nucleus and chloroplast in higher plants, the appointee will investigate the nature of the sequence homology between chloroplast and nuclear DNA.

QUALIFICATIONS: A PhD degree or equivalent qualifications in an appropriate field, (such as biochemistry, plant physiology or biology). Experience within the field of biochemistry of nucleic acids, including recombinant DNA technology and DNA sequencing, would be an advantage.

TENURE: A term of three years, commencing in 1984. Australian Government superannuation benefits are available.

APPLICATIONS: Stating full personal and professional details, the names of at least two professional referees, and quoting reference No A0881, should be directed to:

The Chief
CSIRO Division of Horticultural Research
GPO Box 350
ADELAIDE SA 5001
AUSTRALIA

By July 6, 1984.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W1028)A

AFRC ANIMAL BREEDING RESEARCH ORGANISATION

Positions in Molecular Biology and Cell Culture

ABRO is currently seeking staff to join its newly-founded molecular biology group in Edinburgh. Research objectives are to investigate the potential of new techniques for genome manipulation of higher animals. During an initial period, research is expected to concentrate upon gene transfer into tissue culture cells and experience in genetic manipulation and/or mammalian cell culture would be advantageous.

Scientists with experience of DNA cloning in plasmids, cosmids and phages, sequencing, or DNA introduction into tissue culture cells by micro-injection or transfection are especially invited to apply.

Candidates should possess a good honours degree in molecular biology, biochemistry or a related subject. For appointment at the Higher Scientific Officer level a minimum of two years relevant post-graduate training or experience is expected. Candidates for recruitment at the Senior Scientific Officer level should have at least 4 years relevant post graduate research experience and will normally be expected to possess a PhD or equivalent qualification. Salaries will be according to qualifications and experience on the scales £7,149—£9,561 (HSO) and £8,970—£11,476 (SSO).

Applicants should submit a full curriculum vitae together with the names of two referees to:
The Secretary, Animal Breeding Research Organisation, West Mains Road, Edinburgh EH9 3JQ.

The Agricultural and Food Research Council is an Equal Opportunities Employer. (2264)A

CSIRO
AUSTRALIA
RESEARCH FELLOW/ SENIOR RESEARCH FELLOW DYNAMICAL METEOROLOGIST
$A25,342-$A37,274
DIVISION OF ATMOSPHERIC RESEARCH
MORDIALLOC VIC

GENERAL: CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Atmosphereic Research is of member of the Institute of Physical Sciences.

The Division of Atmospheric Research maintains broadly-based research programs in dynamical meteorology, boundary layer physics, climatology, radiation, cloud physics and atmospheric chemistry. Total staff numbers approximate 130.

The Division's Large Scale Dynamics program is concerned with the study of processes which determine the general circulation of the atmosphere on length scales of 1000 km and greater together with its variability and the climatic consequences of this variability.

DUTIES: To undertake research into the observational analysis and diagnostic aspects of the program and, in collaboration with other members of the group, to contribute to related theoretical and modelling studies of the atmosphere.

QUALIFICATIONS: A PhD degree or equivalent qualifications in the physical sciences with demonstrated research ability and experience in dynamical meteorology. Experience in the analysis and interpretation of atmospheric data is highly desirable.

TENURE: A term of 3 years with the possibility of a further 2 year term. Australian Government superannuation benefits available.

APPLICATIONS: Stating full personal and professional details, the names of at least two referees and quoting reference No A1848, should be directed to:

The Chief
CSIRO Division of Atmospheric Research
Private Bag No 1
MORDIALLOC VIC 3195
AUSTRALIA

By July 6, 1984.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W1030)A

IMMUNOLOGICAL/ MICROBIOLOGICAL RESEARCH
FACULTY OF DENTISTRY — THE UNIVERSITY OF ALBERTA

Applications are invited for a full-time position as a Research Scholar in Immunology and/or Microbiology. The successful candidate must have a research degree in Immunology or Microbiology with an interest in immunochemistry and bacterial ecology related to periodontal diseases. Research will be directed toward characterisation of the interaction of surface components of oral bacteria with secretory antibodies and other secretory glycoproteins and also with serum antibodies. These positions will be dependent upon successful application for a competitive award by an external granting agency. Appointment as Scholar will be on a long-term basis and will enjoy the privileges of the University academic rank at which the appointment is made (Assistant Professor, Associate Professor or Professor level presently $29,720-$41,820; $37,420-$53,658 and from $48,010). The University of Alberta is an equal opportunity employer but in accordance with Canadian immigration requirements this advertisement is directed to Canadian citizens and permanent residents of Canada. **Interested applicants should forward their curriculum vitae, outlines of their research experience and names of three referees to Dr J A Hargreaves, Director of Graduate Studies and Research, Faculty of Dentistry, The University of Alberta, Edmonton, Alberta T6G 2N8. The closing date for applications is July 1, 1984.** (NW583)A

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF PHYSICS
RESEARCH ASSISTANT
AMORPHOUS SEMICONDUCTORS AND DISORDERED SUPERLATTICES

Applications are invited from men and women for the above post funded by SERC, to join an established group intrested in the optical and electronic properties of disordered semiconductors. Systems of particular interest include amorphous silicon, amorphous Si-SiN quantum wells and crystalline GaAs-GaA1As superlattices. Involves the development of novel two beam luminescence techniques, photon deflection spectroscopy for the measurement of weak absorption processes, and the use of a dye laser to measure excitation spectra. Tenable for three years.

Initial salary £7,190-£7,630 a year on Range 1A.

Enquiries to Dr T M Searle/ Professor I G Austin, Department of Physics, the University, Sheffield S3 7RH to whom applications, with the names of two referees, should be sent as soon as possible. Please quote ref R94/g (2253)A

POSTDOCTORAL POSITION available immediately to study 3-dimensional architecture of chromosomes using optical and electron microscopy and computerized, 3-dimensional reconstruction schemes. The candidate should have strong background in computer science/computer graphics and/or macro-molecular crystallographic experience. Send resume with names of 3 references to: Drs David A Agard or John W Sedat, Department of Biochemistry & Biophysics, University of California, San Francisco, California, USA. (NW578)

UNIVERSITY COLLEGE LONDON
COMPUTER PROGRAMMER

Computer programmer with an interest in the nervous system is required to work in a programme on the anatomy and physiology of the visual system in a well equipped laboratory. The successful applicant will be expected to manage the computer systems, maintain existing programmes and develop software to aid research. Programming in several areas, including image processing, graphics and data acquisition is involved. Experience of RSX-IIM, FORTRAN, MACRO-LL, BASIC and 6502 assembler would be an advantage.

Applications, giving the names of two referees, to Professor S Zeki, Department of Anatomy & Embryology, University College London, Gower Street, London WC1E 6BT. (2267)A

WEST VIRGINIA UNIVERSITY
invites applications and nominations for the position of

DEAN OF THE COLLEGE OF OF AGRICULTURE AND FORESTRY AND DIRECTOR OF THE AGRICULTURAL AND FORESTRY EXPERIMENT STATION

The Dean is responsible for all educational, research, extension and service programs of the College and Experiment Station. Qualifications include an earned doctorate, a record of academic and research excellence, a firm commitment to the mission of a comprehensive land grant university and ability to communicate effectively with faculty, staff, students and representatives of commodity groups. Demonstrated administrative leadership is desired.

Applications including a résumé, other supporting material and names of three references will be accepted until July 15, 1984. Nominations and applications should be addressed to: Chairman of Search Committee, School of Dentistry, Medical Center, West Virginia University, PO Box 6305, Morgantown, West Virginia 26506-6305.

West Virginia University is an Affirmative Action, Equal Opportunity Employer. (NW497)A

POSTDOCTORAL POSITIONS (two) available in a leading research group. One for studying mutations in genes for collagens in human genetic diseases, and the other studying gene expressions during mouse embryogenesis. Background in molecular biology helpful but not essential. Interested candidates should send a letter describing research experience with a curriculum vitae and three letters of recommendation to: D J Prockop, MD, PhD, Department of Biochemistry, University of Medicine and Dentistry of New Jersey — Rutgers Medical School, PO Box 101, Piscataway, New Jersey 08854. An Equal Opportunity/Affirmative Action Employer. (NW582)A

THE UNIVERSITY OF SHEFFIELD
POSTDOCTORAL NEUROANATOMIST

Applications are invited from me and women for a

POSTDOCTORAL POSITIOI

funded by the SERC, tenable for fixed term until 15 May 1987 t investigate efferent projections of th superior colliculus. Experience wit neuroanatomical tract tracin techniques essential. The proje offers the opportunity to combi anatomical analysis with behaviour and neuropharmacological tec niques. Initial salary £7190 a year Range IA. Details from Dr Redgrave (0742 78555 ext 6556 Applications, including the names three referees, to the Registrar a Secretary (Staffing), The Universit Sheffield S10 2TN as soon possible. Quote ref: R91/G. (2235)A

Southampton THE UNIVERSITY

NEW BLOOD LECTURESHIP IN GEOLOGY

(Re-advertisement)

Applications are invited for the post of Lecturer in Geology with special emphasis on the geochemistry of fluid-rock interactions as applied to metals and/or hydrocarbons. Applicants with interests in fields such as hydrothermal alteration, petroleum generation and migration, diagenesis, pore fluids, fluid inclusions or stable isotopes will be particularly welcome. This new blood post is intended to reinforce the research interests of the Department in applied geology.

Salary scale: £7,190 × £450 (15) £14,125 per annum (under review). The initial salary will depend on qualifications and experience.

Further particulars may be obtained from Mr D A S Copland, The University, Southampton, SO9 5NH to whom applications (7 copies from United Kingdom applicants should be sent not later than 2 July 1984, quoting reference No. 2039. (2277)A

Senior Positions in Biochemistry and Nerve Cell Biology

The Sandoz Institute for Medical Research invites applications for the positions of Head of Laboratory (one in Biochemistry and one in Cell Biology & Cell Culture) tenable from 1 January 1985.

The Institute will be housed in well-equipped and spacious new laboratory accommodation which is being built within the precincts of University College London, with which strong academic links will be forged. The scientific staff will be up to 25–30 by 1987.

The Institute will embark on a programme of basic research in neurobiology, concentrating initially on mechanisms involved in nociception, with the long-term aim of developing new compounds of potential therapeutic value. Initially there will be six laboratory groups (Medicinal Chemistry, Biochemistry, Neuropharmacology, Cell Biology and Cell Culture, Immunology, Cellular Electrophysiology) which will collaborate on a range of individual projects.

Applicants for both positions need to have proven ability to organize and supervise the running of a research laboratory, and to contribute original ideas to a multidisciplinary research effort. Enthusiasm for interdisciplinary collaboration is essential.

Candidates for the Biochemistry position should have experience in the field of receptor-regulated mechanisms and/or the isolation and characterization of membrane proteins.

Candidates for the Cell Biology position should have general experience in cellular neurobiology and neuronal cell culture techniques, preferably with an interest in the biology of sensory neurons.

Salary negotiable, plus comprehensive benefit package including car. Assistance with relocation if required.

Applications by 16 July 1984, including cv and names of three referees, to Professor H.P. Rang, FRS, Director, Sandoz Institute for Medical Research, Department of Pharmacology, University College London, Gower Street, London WC1E 6BT (Telephone 01-388 5314), from whom further information can be obtained.

SANDOZ

(2302)A

THE UNIVERSITY OF MELBOURNE

RESEARCH FELLOW GRADE 1 (EXTENDIBLE TENURE)

in the

DEPARTMENT OF BIOCHEMISTRY

Applications are invited from people with a background in physical chemistry or physical biochemistry for appointment as Research Fellow 1. The work involves the use of photophysical techniques to characterize the dynamics of cytoskeletal networks and to study proximity relationships in biomembranes. The position is sponsored by the Australian Research Grants Scheme and is for 2 years.

Salary up to $A22,213 per annum and in the range of $A20,991 to $A24,047. For further information please contact Dr W H Sawyer, Department of Biochemistry, University of Melbourne, Parkville 3052, Victoria, Australia.

Further printed information regarding details of application procedure and conditions of appointment is available from the Appointments Officer (Academic), University of Melbourne, or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF.

Applications close on 15 August 1984.

(W1035)A

SCOTTISH CROP RESEARCH INSTITUTE

Invergowrie, Dundee DD2 5DA
Tel: Invergowrie 731
(STD code 082 67)

SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC OFFICER

The Zoology Department requires an SO/HSO to undertake research on mechanisms of plant resistance to insect pests of forage brassicas and soft fruit. The appointee will work closely with plant breeders to examine factors conferring resistance amongst a range of plant breeding material.

Qualifications: A first or upper second class honours degree in an appropriate subject and at least two years' relevant post-qualifying experience which could be in the entomology and/or plant chemistry and may be a course of research and study leading to a PhD degree for appointment to the higher grade.

Salary: SO — £5,682-£7,765, HSO — £7,149-£9,561. Non-contributory superannuation.

The Institute is an equal opportunities employer.

Applications giving full details of age, qualifications and experience together with the names of two referees should be lodged by 9 July, with Mrs J A Shepherd, the Personnel Officer of the Institute, from whom further particulars may also be obtained. (2256)A

BTL

"BUILDING A BIOTECHNOLOGY BUSINESS"

Positions in Environmental and Agricultural Biotechnology

BioTechnica Limited (BTL), a new British biotechnology company affiliated with BioTechnica International Inc. of Cambridge, Massachusetts, seeks scientists for the following positions:

1. **Senior Scientist — Programme Manager for the Pollution Group:** to work on the development of microbial systems for aerobic biodegradation, particularly aqueous treatment systems and decontamination of polluted land. The successful applicant will lead this group and will be concerned both with research and with the development of new products. It is anticipated that candidates will hold a Ph.D. and have had several years post-doctoral experience, probably in industry.

2. **Assistant Scientist:** to work on the growth and downstream processing of microbial products for agriculture. Candidates should preferably hold a good honours degree (or higher degree) in microbiology with a major interest in microbial physiology or fermentation technology.

3. **Research Technician:** to assist the development of micro-organisms for use in agriculture. Candidates should preferably hold a degree in microbiology or have substantial experience in basic microbial culture techniques.

4. **Biochemical Engineer:** To design novel systems for environmental biotechnology. Candidates should preferably hold an M.Sc. degree.

Competitive salaries will be paid and participation in a staff share option scheme is available.

Applications in writing by sending a full CV together with the names of three referees to:

Miss M. Ghosh, Office Manager, BioTechnica Limited, 5, Chiltern Close, Cardiff CF45DL, Wales, U.K. (2266)A

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PATHOLOGY

PARASITE IMMUNOLOGY PROGRAMME MOLECULAR BIOLOGIST

Position immediately available for a postdoctoral scientist up to 32 years old to work on an EEC-supported project until March 1986, with a possibility of further extension.

This new project in the field of onchocerciasis (river blindness) has recently been initiated in a group with a strong background in the immunology and molecular biology of schistosomiasis. Applications are invited from scientists with experience in molecular biology to work on the molecular cloning of DNA encoding antigens of *Onchocerca volvulus* that may be of importance in immunopathology, immunoprohylaxis and immunodiagnosis, and to participate in ongoing studies on schistosomiasis. The project involves close collaborative work with the Institut Pastuer, Lille, and with the MRC Laboratory, Bo, Sierra Leone.

Previous experience in parasitology not required. Salary range University Scale £8,080 to £10,710, depending on age.

Informal enquiries to Dr. A. E. Butterworth or Dr. D. W. Taylor (0223 353492). Formal applications, with curriculum vitae and names of two referees, to Mr. P. Wilburn, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP (0223 64631).

(2233)A

MARIE CURIE MEMORIAL FOUNDATION RESEARCH INSTITUTE

Two Post-doctoral positions in Molecular Biology. The first will involve the application of recombinant DNA technology to the study of cellular gene activation by transforming proteins.

The second will investigate the role of the transformation — associated protein p53 in cell proliferation and transformation. Eukaryotic vectors expressing p53 and a variety of mutants are available for this work.

These three-year appointments are available now and salary will be on the Lecturer Scale.

Apply with C.V. and the names of two referees to Dr. G. Currie, Marie Curi Research Institute, The Chart, Oxted, Surrey (088388-3055). (2163)A

MOLECULAR EVOLUTION/ SYSTEMATICS
Yale University Department of Biology

Yale University's Department of Biology seeks applicants for an assistant professorship in molecular evolution and/or molecular systematics.
Candidates expertise should include both the use of modern DNA technology to investigate either eucaryotic or procaryotic systems and a knowledge of the theory and analytical methods directed toward the study of evolution. Duties associated with this position include teaching of graduate and undergraduate students and management of a strong research program. The appointment will take effect no sooner than July 1, 1985.
Send curriculum vitae, summary of past and present research, and the names of at least three referees to: Executive Secretary, Molecular Evolution Search Committee, Department of Biology, Yale University, P.O. Box 6666, New Haven, CT 06511. Deadline for acceptance of applications is December 15, 1984. Yale University is an Equal Opportunity/ Affirmative Action Employer. (NW588)A

Yale University

WELSH NATIONAL SCHOOL OF MEDICINE
(University of Wales)

DEPARTMENT OF MEDICAL BIOCHEMISTRY

POST-DOCTORAL RESEARCH OFFICER

Applications are invited for the above post in which the successful candidate will apply a group of monoclonal antibodies to the investigation of the clinical significance and molecular biology of complement component C9. The post will be for 3 years and is funded by the Medical Research Council.

Candidates should have, or have submitted, a PhD thesis in biochemistry, immunology or a related discipline. The successful candidate will be working in an active research team headed by Dr A K Campbell engaged in elucidating molecular mechanisms underlying issue injury in immune based disease.

Salary on the scale for University Research and Analogous Staff Grade 1A with commencing salary up to £8,080 for suitably qualified candidates.

Further details from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN (Tel No 0222/755944 Ext 2296) (quoting Ref No C38/4/49) to whom applications in the form of a curriculum vitae with the names and addresses of two referees should be submitted by 6th July, 1984.

(2246)A

THE UNIVERSITY OF MELBOURNE

LECTURER (CONTINUING) in the

DEPARTMENT OF PHYSICAL CHEMISTRY

A vacancy exists in the Department of Physical Chemistry for a continuing appointment at Lecturer level, of a suitably qualified physical chemist. The Department has major research and postgraduate education commitments in areas of colloid and surface chemistry, ultra-fast spectroscopy, photochemistry and electrochemistry. The new appointee would be expected to develop a research programme in one or more of these areas. Opportunity exists to participate in a range of research liaison programmes with other Departments of the University and with CSIRO and the chemical industry.

Salary in the range $A24,353 -$A31,994 per annum. For further information please contact Dr A A Humffray, Chairman of Physical Chemistry, in the University.

Further printed information regarding details of application procedure and conditions of appointment is available from the Appointments Officer (Academic), University of Melbourne, Parkville, Victoria 3052, Australia, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF.

Applications close 30 July 1984.

(W1036)A

THE UNIVERSITY OF LEEDS

DEPARTMENT OF PHYSICS

LECTURER

Applications are invited for the above post in experimental condensed matter physics established under the "New Blood" scheme. The successful applicant will be expected to join the group concerned with experimental and theoretical aspects of metallic glasses, where recent studies have been mainly on their thermal, magnetic, and transport properties. While the appointee will primarily be concerned with the physics of amorphous materials an interest in either new methods of preparation or measurements at millikelvin temperatures would be an advantage.

The age limit for these posts is 35.

Salary on the academic scale for lecturers (£7190-£14125) (under review) according to age, qualifications and experience.

Application forms and further particulars may be obtained from The Registrar, The University, Leeds LS2 9JT, quoting reference number 52/29/D. Closing date for applications 15 June 1984. (2251)A

MANAGER: PROJECT DEVELOPMENT

A position is available in the corporate development program of a US agricultural company. Qualifications are:

1. PhD in Agronomy, Horticulture or related field.

2. Familiar with new technologies in plant genetic engineering, tissue culture and cell biology.

3. Familiar with general agricultural business, seeds and plants.

4. Person with interest in marketing preferred.

Send resume to: Dr Irwin Y E Chu, Corporate Vice President, Research & Development, George J Ball, Inc, PO Box 335, West Chicago, IL 60185 USA. (NW585)A

THE UNIVERSITY OF SHEFFIELD

TEMPORARY LECTURESHIP IN PSYCHOLOGY

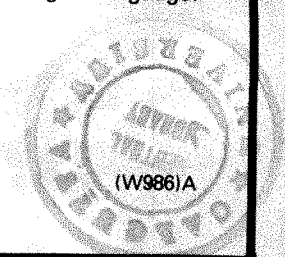
Applications are invited from men and women for the above post, tenable for a fixed term of 3 years from 1st october 1984. The person appointed will have a major responsibility for the teaching of AI and cognition to undergraduates, including the supervision of final year projects using POP 11. Salary scale £7,190-£14,125 a year. Particulars from the Registrar and Secretary (Staffing), The University, Sheffield, S10 2TN, to whom applications (5 copies) including the names and addresses of 3 referees should be sent by 25th June 1984, quoting ref: R97/G. (2236)A

KINGDOM OF SAUDI ARABIA
KING SAUD UNIVERSITY
COLLEGE OF AGRICULTURE
RIYADH, SAUDI ARABIA

Qualified personnel are invited to apply for the following posts of FACULTY MEMBERS (Professors, Associate Professors, Assistant Professors), LECTURERS, DEMONSTRATORS, RESEARCH ASSISTANTS and TECHNICIANS for the academic year 1984 - 1985.

Department	Specialization	Qualification
I. Staff members:		
(1) Food Science	a. Human nutrition metabolism. b. Dairy technology. c. Food analysis.	Ph.D., Teaching experience, Arabic speaking.
(2) Animal Science	a. Poultry nutrition.	-do-
(3) Plant Production	a. Cytogenetics.	-do-
(4) Ag. Economics & Rural Sociology	a. Farm management. b. Marketing of agricultural products.	Ph.D., Teaching experience, Associate or Professors only, Arabic speaking.
(5) Home Economics & Nutrition (Female only)	a. Textiles and costumes.	Ph.D., Teaching experience, Arabic speaking.
II. Lecturers:		
(1) Home Economics and Nutrition (Female only)	a. Plant production (ornamentals) b. Plant production (field crops).	M.Sc., Teaching experience, Arabic speaking.
(2) Plant Production	a. Plant production (vegetable crops).	-do-
(3) Animal Production	a. Animal production	-do-
III. Demonstrators:		
(1) Food Science	a. Fish and meat technology. b. Food microbiology. c. Food analysis.	B.Sc. "B" average or higher, past experience in teaching, research is preferred, Arabic speaking.
(2) Plant Production	a. Horticulture (vegetable crops).	-do-
(3) Agricultural Engineering	a. Agric. Engineering.	-do-
IV. Research Assistants:	a. Food microbiology. b. Food chemistry and analysis. c. Horticulture (Pomology). d. Field Crops (Breeding).	Preferably M.Sc. with research experience in the field of specialization.
V. Technicians:	a. Human nutrition. b. Cereal technology. c. Analytical chemistry in the field of food science.	Experience in field of work, conducting and operating laboratory equipment. Experience and training in operating food and nutrition analyses equipment preferably IR, AAA, AA, GLC, HPLC. Good knowledge of English language.

Please apply to:
Dean, College of Agriculture,
King Saud University,
P.O. Box 2460,
Riyadh 11451,
Kingdom of Saudi Arabia.



(W986)A

Opportunities in Pharmaceutical Development

Pathology Division *Ware, Herts*

Glaxo Group Research is Britain's leading pharmaceutical research organisation, with a distinguished reputation for developing safe and effective medicines. Due to an expanding workload, two positions have arisen in our Pathology Division.

Cytogeneticist

We require a good honours graduate to join our team of Genetic Toxicologists. You will be involved in cytogenetic analysis including metaphase chromosome studies of mammalian cells. You will be expected to assist in the preparation of the specimens for analysis and in the assessment of results. The laboratories are purpose built to the highest standard and equipped with the latest computer technology.

Applications are invited from graduate cytogeneticists with some experience of metaphase analysis.

Reproductive Toxicologist

We require a graduate to be a study supervisor in our Toxicology Department working in the team assessing the safety of new drugs when used in pregnancy.

You will be involved in the implementation of reproductive toxicology studies and ensuring the correct execution of the studies according to GLP requirements.

You will be expected to be fully aware of the status of each study, as it proceeds, by collating and correlating data generated by the department's dedicated computer system.

Candidates should possess a relevant good honours degree together with a sound knowledge of animal anatomy. Experience gained in this field of work would be a distinct advantage.

We offer a competitive salary in the range £6,175-£10,770. Your starting salary will reflect your experience. Other benefits of a highly successful company include participation in Glaxo Group profitability, non-contributory pension scheme, an active sports and social club and, where appropriate, assistance with relocation to this pleasant area of Hertfordshire.

Please write or telephone for an application form, stating which vacancy interests you to: Mr. D. Latter, Personnel Officer, Glaxo Group Research Ltd., Ware, Herts. SG12 0DJ. Tel: Ware (0920) 3993.

Glaxo *Group Research Ltd.*

(1954)A

University College of Swansea

Part-Time Teaching Assistant

Applications are invited for the vacancy of **Part-time Teaching Assistant** in the **Department of Zoology.** Applicants should have a Ph.D. degree and a research interest in the cellular effect of heavy metals on fish reproductive systems.

The appointment, which will be for one year from October 1, 1984, will be on a scale up to £4,265 per annum.

Further particulars and application forms (2 copies) may be obtained from the Registrar, University College of Swansea, Singelton Park, Swansea SA2 8PP, to which office they should be returned by Friday, June 29, 1984. (2278)A

POSTDOCTORAL FELLOW/ASSISTANT PROFESSOR (PhD or MD) in molecular biology to join basic gastrointestinal research laboratory. Applicant should have strong background in molecular biology. Candidate will join group with ongoing interest in malignant transformation, intestinal cell function and transport, molecular biology involving viral hepatitis and hepatoma, and studies of oncogenes in gastrointestinal malignancy. Individual will be encouraged to conduct independent research but will be expected to participate in collaborative projects. Send curriculum vitae, bibliography, references, and statement of research interests to: Dr Kurt J Isselbacher, Chief, Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA 02114. An Equal Opportunity Employer.

(NW579)A

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nature classified

LONDON OFFICE
Keith Walesby
4 Little Essex Street
London WC2R 3LF
Tel: 01 240 1101
Telex: 262024

NEW YORK OFFICE
Miss Gene Fein
15 East 26 Street
New York, NY 10010
Tel: (212) 689 5900

TORONTO OFFICE
Peter Drake Associates
32 Front Street West
Suite 200, Toronto,
Ontario M5J 1C5
Tel: (416) 364 1623

Public Service Commission of Canada | **Commission de la Fonction publique du Canada**

Research Scientist, Earth Structure Studies

Energy, Mines and Resources
Earth Physics Branch
Ottawa, Ontario

We require a geophysicist/geologist to conduct field investigations and research studies into the nature and structure of major geological features throughout the Canadian landmass. Emphasis will be on the application of deep seismic reflection techniques, including vibrosesis, and regional three-dimensional seismic refraction methods. Other geological and geophysical methods will be incorporated as necessary. Field investigations will be carried out in all parts of Canada, from the east and west coasts to the Arctic Ocean Basin. Research studies will demand extensive use of state-of-the-art computer-based processing and international methods.

You require graduation with a doctorate degree from a recognized university in geophysics, geology, physics or a related field, or a lesser degree with evidence of research experience and productivity equivalent to that of a doctorate degree. Extensive experience in the processing and interpretation of multifold seismic reflection data is also required. Knowledge of either English or French is essential.

We offer a salary ranging from $26,834 to $49,913 commensurate with your qualifications and experience. Effective 23 June 1984, the revised salary range will be $28,176 to $52,409.

Forward your application form and/or résumé, quoting reference number S-84-31-5523-JG47(G26), to:
Joan Girling (613) 593-5331
Public Service Commission of Canada
Ottawa, Ontario K1A 0M7

Closing date: 31 August 1984 (NW592)A

The Public Service of Canada is an equal opportunity employer Canada

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POSTDOCTORAL RESEARCH POSITION: Available immediately for recent PhD or MD with a strong background in molecular biology/genetics of animal viruses. Candidates with excellent academic records, some experience in DNA technologies including cloning and a strong interest in tumor virology will be given preference. Please send résumé, abstract of the thesis along with other evidence of scholarly productivity, and the name and telephone numbers of three referees to: Nurul H Sarkar, PhD, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, NY 10021, USA. (NW541)A

POSTDOCTORAL POSITION: protein chemistry and/or immunochemistry background. This person will provide the biochemical expertise for a small team developing synthetic membrane bound ion-channel receptors. Experience with cross-linking or affinity labelling and lipid membranes would be helpful. This position is for an independent, self-motivated scientist. It is an academic research position in a major university in western New York. Box NW581 c/o Nature, 15 East 26 Street, New York, NY10010. (NW581)A

BIOCHEMIST to direct research related to function of enzymes connected with certain immunodeficiency diseases. Strategy requires the cloning of the corresponding genes. PhD/Biochemistry and 3 years postdoctoral experimentation and research involving nucleic acid cloning techniques, protein biochemistry and enzymology. Salary $36,000 per year. Job site: South San Francisco. Send this ad and your résumé to Ad No 9075, PO Box 865, Sacramento, CA 95804 not later than June 22. (NW584)A

Faculty of Earth Sciences, King Abdulaziz University, Jeddah, Saudi Arabia,

invites applications for a faculty position for academic year 1984-85 starting September 1984 at

Associate professor to Professor level in the Department of Geochemistry

to develop and coordinate research in the field of exploration geochemistry and to be involved in the undergraduate and postgraduate teaching programmes. Excellent geochemical laboratory facilities are available.

Ideal candidates would have a Ph.D. degree in geochemical prospecting or very related field with long experience in exploration geochemistry according to which salary would be determined.

Conditions of employment include:

* excellent salary untaxed at source
* free furnished accommodation
* two months annual leave with return air tickets to place of origin

Apply with complete c.v. and list of publications to the Dean, Faculty of Earth Sciences, King Abdulaziz University, P.O. Box 1744, Jeddah, Saudi Arabia.

Applications must reach the Faculty of Earth Sciences not later than one month from date of advertisement.

(W1026)A

2 POST DOCTORAL RESEARCH POSITION IN IMMUNOCHEMISTRY

open immediately at Institut National Recherche Agronomique, at Tours, to study proteins involved in cell adherence. Salary between 12 and 14 000 $ per annum.

Send curriculum vitae and names of references to Dr A. Paraf, Station de Virologie INRA, 78850 Thiverval-Grignon, France.

(W1039)A

DEPARTMENT OF BIOLOGY

Applications are invited for a regular full-time Assistant professor position in Comparative Microanatomy effective 1 July 1985 subject to final budgetary approval. Candidates are expected to have a Ph.D., preferably with post-doctoral experience. While the Biology Department is receptive to applicants from a wide range of backgrounds in Zoology, preference will be given to an candidate whose interests complement those of present faculty and who would make substantial use of our electron microscope facilities. The successful applicant would be expected to develop a vigorous research program and to supervise graduate students. Participation in appropriate cellular, animal histology and animal developmental undergraduate courses would be expected. The Assistant professor salary floor is $26,465. **Applicants should submit, not later than July 31, 1984, a curriculum vitae, personal statement of teaching and research interests, and arrange to have three letters of recommendation sent to:**

Dr J.E. McInerney, Chairman
Department of Biology
University of Victoria
Victoria, B.C. V8W 2Y2

Canadian immigration regulations now require the University to assess applications from Canadian citizens and permanent residents of Canada before assessing applications from other persons. The University of Victoria offers equal employment opportunities to qualified male and female applicants.

(NW580)A

UNIVERSITY OF NEWCASTLE UPON TYNE

CHAIR OF AGRICULTURAL BIOLOGY

Applications are invited for the Chair of Agricultural Biology which has become vacant through retirement. Preference will be given to candidates with interests in applied ecology, including pest and disease management and other aspects of crop improvement. The appointment will carry the headship of the Department of Agricultural Biology for an initial period of five years.

The appointment will be effective from 1 October 1984 or as soon as possible thereafter. Salary will be at an appropriate point on the range for professorial staff which commences at £17,275.

Further particulars may be obtained from the Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU, with whom applications (15 copies), giving the names of not more than three referees, must be lodged not later than 27 July 1984. Candidates from outside the British Isles may submit one copy only.

(2257)A

UNIVERSITY OF BRISTOL MEDICAL SCHOOL

ANIMAL SUPERINTENDENT

Applications are invited for the post of Animal Superintendent for the Medical School. The position involves responsibility for the running of the animal house and applicants should be fully conversant with laboratory animal husbandry and the organisation of a modern animal facility. Since the staff of the animal house number about 20 and belong to at least eight different departments, managerial experience is essential. Fellowship of the Institute of Animal Technicians, whilst not mandatory, would be a desirable qualification. The appointment will be at the level of Grade 8C and in the salary range of £9351 to £10,168 according to experience. (Scales under review). Applications, together with a curriculum vitae, and the names and addresses of two referees, should be sent not later than 20th June to the Personnel Office, Senate House, University of Bristol, from whom particulars may be obtained. (Please quote reference PHC).

(2275)A

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF GENETICS AND BIOMETRY

POST-DOCTORAL RESEARCH ASSOCIATE

Required for a three year appointment to participate in the application of new mathematical techniques to the theory of species formation, and analysis of field data, in spatially distributed populations.

The post would be suitable for applicants with a background in applied maths/physics or a biologist with strong quantitative talents.

Further details from, and applications, cv and names of two referees to, Dr N H Barton, Department of Genetics, University College London, 4 Stephenson Way, London NW1 2HE. (2298)A

UNIVERSITY OF OXFORD

Research laboratory for Archaeology

RESEARCH ASSISTANT IN CHEMISTRY

A post at post-graduate or post-doctoral level is available in the Radiocarbon Accelerator Unit for two years with probable extension to at least four years. The RA will manage the non-routine chemical work of the Unit, and initiate research into the isolation of pure materials from archaeological samples and in the preparation of novel types of ion source target. This work is directed towards improving the accuracy and extending the time-range of radiocarbon dating of micro-samples.

Applicants should have a degree in chemistry and further experience would be an advantage. The salary will be on the 1B scale (£6,625-£10,370 approx) or on the 1A scale (£7,550-£12,195 approx) according to experience and qualifications. Applications should be made to Dr R E M Hedges, Director, Radiocarbon Accelerator Unit, Research Laboratory for Archaeology, 6 Keble Road, Oxford, from whom further details may be obtained. (2234)A

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF BIOCHEMISTRY

LECTURER IN BIOCHEMISTRY/ MOLECULAR BIOLOGY

Candidates should possess a PhD in Biochemistry or Molecular Biology and postdoctoral experience in the application of molecular biology to eukaryotic systems Permanent appointment, following successful completion of the initial probationary period. Salary range £7,190 — £14,125 per annum plus £1,186 London Allowance, according to age and experience. Starting date will be 1 October 1984 or by agreement.

Person appointed will be expected to develop a strong research programme in an area of eukaryotic molecular biology, preferably related to metabolic control, and to contribute to the teaching of undergraduates and MSc students.

Applications, including full cv, the names of two academic referees and a brief indication of future research interests, to Profesor B R Rabin, Department of Biochemistry, University College London, Gower Street, London WC1E 6BT. Closing date: 13 July 1984. (2299)A

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
DIVISION OF PARASITOLOGY

Applications are invited for a

POSTDOCTORAL POSITION

in the Division of Parasitology to work with Dr K.N. Brown on the immunogenicity of erythrocytes infected with malaria parasites. The appointment will be for 3 years from 1st October 1984. Experience in membrane biochemistry and/or immunology would be advantageous.

The salary will be in the range £8,530 – £10,250 plus £1,186 London allowance, with superannuation provision.

Applications, together with curriculum vitae, research experience and interests and the names of two professional referees should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA before 29th June 1984, quoting ref: P/ 6/ R.

(2289)A

UNIVERSITY OF EDINBURGH
DEPARTMENT OF MEDICINE
(Western General Hospital)

LECTURESHIP
(Non-Clinical)

Applications are invited from suitably qualified science graduates for appointment to a post of Lecturer in the Department of Medicine, Western General Hospital which is currently available. This non-medical post will provide for the permanent appointment in a clinical department of a Scientist who will be expected to undertake research in endocrinology with particular reference to endocrine aspects of hypertension. Candidates should have several years postdoctoral research experience. This should preferably inlcude experience of radioimmunoassay. Previous research in the field of the renin-angiotensin-aldosterone system would be an advantage.

Salary will be at an appropriate point on the non-medical scale £7,190 – £14,125 per annum according to age, qualifications and experience.

Informal enquiries may be made to Professor C R W Edwards (Tel: 031-332 2525 ext 4687).

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL with whom applications (8 copies) together with the names and addresses of 3 referees should be lodged not later than 6th July, 1984.

Please quote reference no 1139.

(2295)A

The Queen's University of Belfast

RESEARCH OFFICER IN THEORETICAL ATOMIC PHYSICS

Department of Applied Mathematics and Theoretical Physics

The above post is to support research in theoretical atomic and molecular physics and astrophysics which makes extensive use of a Departmental VAX 11/750, a University ICL dual 2988 and a CRAY-1S at the University of London Computer Centre in developing and running large FORTRAN programs. The person appointed will be expected to collaborate with members of staff in this research and to take administrative responsibility in helping to organise this varied computational work. In addition, he or she would be expected to carry out some teaching, particularly on research and computing.

Candidates, preferably holding a PhD degree, must have some experience in computing and have a good knowledge of quantum theory.

Salary scale: £7,191-£11,161, initial placing depending on age, experience and qualifications.

Applications, including curriculum vitae and the names and addresses of two referees should be sent to the Personnel Officer, The Queen's University of Belfast, Northern Ireland, BT7 1NN. Closing date: 18th June, 1984. (Please quote Ref. 84/N).

(2239)A

FACULTY POSITION
MOLECULAR GENETICIST

The Division of Clinical Immunology and Rheumatology is inviting applications for a tenure-track position at the assistant/associate professor level. A strong commitment to research excellence will be the main criterion of selection. Training in molecular biology is required and research interests in the areas of immunology or mechanisms of inflammation preferred. Candidates must have demonstrated research ability and will be expected to establish a strong independent research program. preference will be given to candidates holding an MD degree. opportunities for participating in graduate programs of Basic Science Departments are available. Curriculum vitae, statement of research goals and interests and names of three referees should be sent by August 1, 1984, to: John E Volanakis, MD, Molecular Geneticist Search Committee, Division of Clinical Immunology and Rheumatology, University of Alabama in Birmingham, Birmingham, Alabama 35292. An Equal Opportunity/Affirmative Action Employer. (NW568)A

CSIRO
AUSTRALIA
BIOCHEMIST/ MOLECULAR BIOLOGIST
$A32,367-$A43,941
DIVISION OF PLANT INDUSTRY
CANBERRA ACT

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Plant Industry is a member of the Institute of Biological Resources.

FIELD: Molecular Biology of Symbiosis.

GENERAL: The Division of Plant Industry conducts research on problems fundamental to plant biology and agricultural production. It has extensive research programs in biochemistry and physiology of biological nitrogen fixation concentrating on the root nodule systems of legumes but also including studies of saprophytic diazotrophic bacteria. There is also an active program on molecular aspects of plant and microbial genetics. It is planned to extend research into the molecular biology of the legume symbiosis with some initial concentration on aspects related to energy-yeilding processes.

DUTIES: To initiate research on the molecular biology of the symbiotic association between legumes and their root nodule bacteria.

QUALIFICATIONS: A PhD degree or equivalent qualifications with extensive experience in molecular biology and biochemistry, especially in the fields of nitrogen fixation and/or bioenergetics.

TENURE: Subject to a satisfactory probation period, an indefinite appointment will be made. Australian Government superannuation benefits are available.

APPLICATIONS: Stating full personal and professional details, the names of at least two referees and quoting reference No A4636, should be directed to:

The Chief
CSIRO Division of Plant Industry
GPO Box 1600
CANBERRA ACT 2601
AUSTRALIA

By July 6, 1984.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W1027)A

United Medical & Dental Schools of Guy's & St. Thomas's Hospitals

NEW BLOOD LECTURESHIP
Prince Philip Research Laboratories
Molecular Genetics

A molecular biologist is required to join a research group in the Prince Philip Research Laboratories at Guy's working on inherited human diseases. The group is part of a multi-disciplinary research unit, with very good laboratory facilities, scientific information service, and other background facilities. The Department contains the South-East Thames Regional Genetics Centre. The molecular genetics group also has well established collaborative arrangements with a number of leading research centres.

The applicant should have experience of recombinant DNA work, e.g. gene cloning, expression or structural analysis. Experience in mammalian cell genetics, gene mapping or linkage analysis would be an advantage.

Applicants wishing to visit the Department should contact Professor M. Bobrow or Dr. F. Giannelli (01-407 7600 ext 2330).

Appointment to this post will be for an initial period of three years. Applicants should not exceed 35 years of age and must not currently hold a permanent UK university appointment.

Salary in range £8,080-£14,125 plus £1,186 London Allowance and Superannuation.

Application forms are obtainable from and should be returned to the Dean, Guy's Hospital Medical School, London Bridge, SE1 9RT not later than 21st June 1984.

(2245)A

THE ROYAL SOCIETY
APPOINTMENT OF EXECUTIVE SECRETARY

A successor is being sought for Dr R.W.J. KEAY, C.B.E., who is retiring at age 65 in mid-1985.

The Executive Secretary is the permanent head of the secretariat with about 100 staff and is responsible under the Officers and Council for the conduct of the administrative business of the Society.

The Society's responsibilities, supported by an annual income of about £8 *million*, cover a wide range of national and international scientific activities. The Executive Secretary is personally involved in all of these and is called upon to represent the Society in many ways at home and abroad.

Applications are sought from persons preferably between 45 and 55 years of age who have had significant personal involvement in scientific research and substantial experience in a relevant administrative post. The Executive Secretary is expected to live in a spacious flat within the Society's premises. The salary is related to that of university professors. The Society has its own pension scheme, with 65 as the normal retirement age.

Those interested should write **in confidence** enclosing a brief **curriculum vitae** and the name of two referees to: SIR JOHN MASON, C.B., F.R.S., Treasurer of the Royal Society, CENTRE FOR ENVIRONMENTAL TECHNOLOGY, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, 48 PRINCE'S GARDENS, LONDON, SW7 1LU by 13 July 1984. (2244)A

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF BIOSCIENCE AND BIOTECHNOLOGY

"NEW BLOOD" LECTURESHIP IN FOOD SCIENCE DIVISION

Applications are invited from candidates under 35 years of age for a lectureship in Food Science. Preference will be given to candidates with postdoctoral research experience in food-related topics. Experience in one or more of the following areas will be an advantage:- biopolymers, rheology, food preservation, food microbiology, food processing and control systems.
Salary will be on the Lecturer Scale (£7,190-£14,125 pa). USS benefit.
Further particulars and application forms (quote ref: 30/84) are available from Staff Office, McCance Building, 16 Richmond Street, Glasgow G1 1XQ.
Closing date for applications: 29 June 1984. (2240)A

United Medical & Dental Schools of Guy's & St. Thomas's Hospitals
NEW BLOOD LECTURESHIP

Department of Chemical Pathology

A molecular biologist with research experience is required in the Department of Chemical Pathology based at Guy's. The post offers the opportunity to work in association with a team involved in the development of malarial vaccines using recombinant DNA Technology. Applicants should submit an outline of research proposals relating to the investigation of human pathology.
Applicants wishing to visit the Department should contact Professor S Cohen (01-407 7600, ext 3365).
Appointment to this post will be for an initial period of three years. Applicants should not exceed 35 years of age and must not currently hold a permanent UK university appointment.
Salary in range £8,080 — £14,125 plus £1,186 London Allowance and Superannuation.
Application forms are obtainable from and should be returned to the Dean, Guy's Hospital Medical School, London Bridge, SE1 9RT not later than 21st June 1984. (2172)A

UNIVERSITY COLLEGE DUBLIN

Applications are invited by the Governing Body of the College for the following full-time statutory appointment:

PROFESSORSHIP OF BOTANY

Prior to application, further information (including application procedure) may be obtained from the Secretary and Bursar, University College, Belfield, Dublin 4. Telephone enquiries: 693244, ext. 431.

The closing date for receipt of completed applications is Thursday, 12th July 1984. (2273)A

RESEARCH POSITION in Paris Laboratory

with possibility of travel and relocation in UK and U.S.A.

Suitable for young independent, ambitious scientist with strong friendly personality.

Should be imaginative, creative and capable of working on unorthodox ideas. Some conversational French necessary.

Training: biochemistry (nucleoproteins), molecular biology, (genetic engineering). Good biology/immunology background.

The work is supported by a British biotechnology company. Salary £8-13,000 p.a.

Please send CV, hand written letter and photo to Box W1038, c/o Nature, 4 Little Essex Street, London WC2R 3LF. (W1038)A

UNIVERSITY OF DÜSSELDORF

ASSOCIATE PROFESSORSHIP (C3) IN MICROBIOLOGY

Applicants are invited for the above position in the Institute of Microbiology, University of Düsseldorf. Candidates should be working in the field of molecular genetics of yeast or filamentous fungi to enable a close cooperation with existing research programs in the Institute. Additional funds and positions are available.

Further information can be obtained from Prof Dr C P Hollenberg, Tel 0211-3114720.

Applications, including curriculum vitae, a summary of research results and the names and addresses of three referees, should be sent to:

The Dekan der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Düsseldorf, Universitätsstraße 1, D-4000 Düsseldorf 1 (FRG).

Closing date 12 July 1984. (W1022)A

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Head of Pharmacology

KENT

The Wellcome Foundation Limited is a major group of pharmaceutical and chemical companies with headquarters in the UK and a turnover close to £700m.

This is a key senior appointment reporting to the Director of Therapeutic Research Division. The department is active on a wide variety of forward-looking research projects and comprises some 60 personnel – including scientists, technicians and clerical/secretarial staff.

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Candidates must be able to demonstrate a substantial scientific and managerial track record in a relevant field and have the maturity to promote and develop scientific excellence through personal leadership and motivation. As a senior scientist, the successful candidate must also be capable of contributing to the general debate on scientific research methodology objectives and maintaining a contemporary scientific perspective in this important area of research.

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Please write with full c.v. to
Alan Murdoch, Personnel Officer,
The Wellcome Research Laboratories,
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(2108)A



Wellcome

UNIVERSITY OF NOTTINGHAM

LECTURER IN ZOOLOGY

Applications are invited for the post as Lecturer in Zoology which will become vacant on 1st September 1984.

Candidates will be expected to have research experience in Neuro Chemistry.

Salary will be within the range £7,190 — £14,125 per annum.

Application forms and further particulars, returnable no later than 25th June 1984, may be obtained from Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Ref No: 951. (2288)A

FELLOWSHIPS

POSTDOCTORAL FELLOWSHIP IN ONCOGENE RESEARCH

Friedrich Miescher-Institut, Basel, Switzerland

Applications are invited for an internally funded position, of three years maximum duration, to work on the role of oncogenes in human cancer. The Institute provides outstanding facilities in a multidisciplinary basic research environment.

Candidates with a background in molecular biology should write to: Dr Christoph Moroni, Friedrich Miescher-Institut, P.O.B. 2543, CH-4002 Basel, Switzerland. (W1025)E

THE UNIVERSITY COLLEGE OF WALES ABERYSTWYTH

DEPARTMENT OF ZOOLOGY

POST-DOCTORAL FELLOWSHIPS

Applications are invited for two Post-Doctoral Fellowships in parasite Biochemistry, one to work on the cytochrome chain asssociated with peroxidases of parasitic nematodes, the other on the carbohydrate metabolism on Onchocerca. Informal enquiries to professor J Barrett (0970 3111, ext 3259). Salary on the 1A Range for Research and Analogous Staff.

Applications with curriculum vitae and the names and addresses of two referees should be sent as soon as possible to the Staffing Officer, The University College of Wales, Old College, King Street, Aberystwyth, SY23 2AX from whom further particulars may be obtained, (Tel 0970 3177, ext 207). (2262)E

THE ROCKEFELLER UNIVERSITY POSTDOCTORAL FELLOWSHIPS IN MOLECULAR PARASITOLOGY

Applications are invited for two postdoctoral positions to study gene structure and regulation in human malaria parasites and African trypanosomes. The research program of the Laboratory of Molecular Parasitology embraces the study of membrance and genetic phenomena in parasitic protozoa. Applicants should be able to show evidence of achievement and potential in molecular or cellular biology. Experience with parasites could be an advantage but is not essential. Applications, enclosing a full CV and names of at least two referees, should be submitted to: Dr George A M Cross, The Rockefeller University, 1230 York Avenue, New York, New York 10021.

An equal opportunity employer. (NW586)E

THE LONDON HOSPITAL MEDICAL COLLEGE (University of London)

ARC BONE AND JOINT RESEARCH UNIT

RESEARCH FELLOW

An Arthritis and Rheumatism Council Fellowship is available until March 1986 to study local variations in biosynthetic activity in human articular cartilage.

Applications with CV and the names of two referees to Professor H L F Currey, Bone and Joint Research Unit, The London Hospital Medical College, Turner Street, London E1 2AD.

Further information may be obtained from Dr J R Archer, Tel 01-247 8939. (2276)E

ANIMAL DISEASES RESEARCH ASSOCIATION

POSTDOCTORAL FELLOWSHIP

Applications are invited from scientists with experience in molecular biology for a Postdoctoral Fellowship tenable in the Moredun Research Institute, Edinburgh, from August/September 1984 to May/June 1985. The appointee will be required to contribute to one or more current projects on microbial pathogens of ruminant livestock. Salary, for the period of the Fellowship will be within the range £8,000-£10,000 per annum dependent upon qualifications and experience. Further details may be obtained on request. Applications, including Curriculum Vitae and names of 2 referees should be submitted by July 5th to The Secretary, Animal Diseases Research Association, Moredun Institute, 408 Gilmerton Road, Edinburgh, EH17 7JH. (2263)E

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF MICROBIOLOGY

POST DOCTORAL RESEARCH FELLOWSHIP

Applications are invited for this A & FRC funded post to work on the biochemical basis of enhancement of disease resistance in rice by dichlorocycloprotanes. Experience of biochemical aspects of plant-parasite relationships an advantage.

Post tenable for up to ten months from 1st July 1984 or as soon as possible thereafter.

Salary on the Research Fellow 1A scale £7,190 to £11,615 plus superannuation.

For further particulars phone 021 472 1301 ext 2559, quoting reference M4.

No formal application form. Three copies of application, including full curriculum vitae and naming three referees to Assistant Registrar, (Science and Engineering), PO Box 363, Birmingham B15 2TT, by 22 June 1984. (2242)E

continued on page 20

ASSISTANTSHIPS

UNIVERSITY OF CAMBRIDGE

MARINE GEOCHEMISTRY

TWO RESEARCH ASSISTANTSHIPS IN ESTUARINE PROCESSES

The posts are for work on the study of variations in the chemistry of suspended particles in estuaries, especially the role of benthic fluxes and processes at the turbidity maximum in chemical scavenging, and will be in collaboration with workers from the Institute for Marine Environmental Research, Plymouth, the marine Biological Association, Plymouth, and Woods Hole Oceanographic Institution.

Post-doctoral level post salary scale £7,190-£8,080. Post-graduate level post salary scale £6,315-£7,190; both to a maximum of three years, staring 1st October 1984.

For the PDRA post, experience in trace metal geochemistry and/or isotope geochemistry, especially solid-source mass spectrometry would be an advantage. The PDRA will be based at Cambridge but may be expected to take part in field work.

For the PGRA post, experience in oceanographic or estuarine field work and in chemical techniques of water and sediment analysis would be an advantage. The PGRA will be expected to be based at Plymouth for the first part of the project and at Cambridge for the second part.

Applications, including a curriculum vitae and the names of two referees should be submitted to Dr H E Elderfield, Bullard Laboratories, Department of Earth Sciences, Madingley Road, Cambridge CB3 0EZ, by 13 July 1984. (2284)P

QUEEN MARY COLLEGE University of London

MATHEMATICAL SCIENCES — THEORETICAL ASTRONOMY UNIT

Applications are invited for an SERC-funded

POSTDOCTORAL RESEARCH ASSISTANTSHIP

for 2 years from 1 October, for work with Dr M Rowan-Robinson on infrared sources detected by the IRAS satellite. The successful applicant will be expected to work on identification of IRAS sources, to initiate and carry out ground-based observational programmes in collaboration with other groups, and to carry out analyses of relevant IRAS data. Experience of ground-based observations at optical or infrared wavelengths, and of computer programming, an advantage. The College has active groups in several branches of astronomy, ranging from cosmology to interstellar molecules and solar physics.

Salary in range (under review) £8,376-£9,716 pa (including London Allowance). Please apply in writing, enclosing cv and names of 2 referees, to the Senior Personnel Officer, Queen Mary College, Mile End Road, London E1 4NS, by 22 June. (2247)P

CAVENDISH LABORATORY UNIVERSITY OF CAMBRIDGE

POLYMER PHYSICS GROUP

AGRICULTURAL AND FOOD RESEARCH COUNCIL (AFRC)

(Food Physics Division)

A number of graduate and post-doctoral Research Assistantships are being offered as part of the link programme with AFRC being set up at the Cavendish to investigate the physics of macromolecules The positions are for three years in the first instance (starting 1 September 1984 or as soon as possible thereafter) and will involve both experimental and theoretical projects. Applicants with training in Physics or Physical Chemistry will be considered.

Applications should be sent to: Mr J Deakin, Secretary, Cavendish Laboratory, Madingley Road, Cambridge CB3 OHE, quoting ref PPG. (2237)P

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PLANT PHYSIOLOGIST/ BIOCHEMIST. (Aged 35). British. BSc, MSc, PhD. 6 years African university lecturing experience. 10 publications. Some journal editorial experience. Broad background from plant tissue culture to crop physiology. Seeks lecturing/research work anywhere in Europe. Box W1032 c/o Nature, 4 Little Essex Street, London, WC2R 3LF. (W1032)B

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The deadline for abstracts is extended to June 15, 1984. Abstracts can be typed on a good quality paper sheet (6.7 inches height & 4.9 inches width) with 5 copies.

Please select a topic among the following:

1. Polyunsaturated fatty acids: biosynthesis and transport
2. Prostanoids
3. Lipoxygenase products
4. Platelet activating factor
5. Free radicals
6. Contact system proteins and kinins
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8. Tissue degrading enzymes
9. Acute phase reactants
10. Neutrophil activation and chemotaxis
11. Macrophage activation and macrophage-derived factors
12. Defects in phagocyte functions
13. Mast cells and inflammation
14. Immediate and delayed type hypersensitivity
15. Cellular interactions
16. Haemostasis and thrombosis
17. Vascular injury
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(W1040)Q

STUDENTSHIPS

IMPERIAL COLLEGE UNIVERSITY OF LONDON

DEPARTMENT OF PURE AND APPLIED BIOLOGY

MRC RESEARCH STUDENTSHIP

Applications are invited from candidates with, or expecting to qualify with, a first class honours or 2(1) degree, for a 3 year studentship to study the biochemical/molecular organisation of the sexual stages of the malarial parasite plasmodium falciparum. The student will be expected to have a sound working knowledge of biochemical techniques, will join an active research group based at the department's field station at Ascot, and will be registered for a higher degree in the University of London.

Applications with CV and the names of two referees to Dr R E Sinden, Imperial College Field Station, Silwood Park, Ascot, Berks SL5 7PY (Closing date May 31st 1984).

(2279)F

QUEEN MARY COLLEGE (University of London)

SCHOOL BIOLOGICAL SCIENCES

RESEARCH STUDENTSHIP

Applications are invited for an SERC studentship to work on a project involving the study of tetrachromatic vision in fresh-water fish. Training will be given in spectrophotometric and electrophysiological techniques. Applicants should possess a first or upper second class degree in biological sciences with a physiological background.

Applications, including curriculum vitae, and the names of two referees should be sent to Dr J K Bowmaker, School of Biological Sciences, Queen Mary College, Mile End Road, London, E1 4NS. (Tel: 01-980 4811, ext 4110).

The School of Biological Sciences has three SERC studentships available for work on a wide range of biological topics. Further details available from the above address.

(2272)F

UNIVERSITY OF ABERDEEN

DEPARTMENT OF ZOOLOGY

has been awarded three

SERC STUDENTSHIPS

starting in October 1984. Applications are invited from students with good Honours Degrees and from those expecting to graduate shortly, and who wish to carry out research in the following areas:

Cell Biology, Experimental Parasitology, Insect Endocrinology, Mammalian Reproductive Energetics, Marine Biology, Neurobiology.

Enquiries and applications should be addressed to Professor W Mordue, DSc, Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen AB9 2TN. Tel No (0224) 40241, ext 6427.

(2300)F

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF BIOCHEMISTRY

SERC — CASE STUDENTSHIP

An SERC-CASE Studentship in collaboration with Dr K Barratt-Bee (ICI Pharmaceuticals) is available to work on the "Transport of purines and pyrimidines in the yeasts Candida albicans and Torulopsis glabrata". The object of the project is to analyse in detail the specificity of the system and mechanism by which these compounds are transported across the plasma membrane. It is to be hoped that information of this type will help in the design of antifungal drugs which like 5-fluorocytosine can be transported illicitly into pathogenic fungi.

Further information can be obtained from: Dr D Kerridge, Department of Biochemistry, Tennis Court Road, Cambridge, CB2 1QW. Telephone: 351781 ext 246.

(2230)F

JOHN INNES INSTITUTE

Colney Lane, Norwich NR4 7UH

RESEARCH STUDENTSHIP

Applications are invited for a three year research studentship to study the physiology/biochemistry of the relationship between photosynthesis and respiration in higher plants.

Applicants should have, or expect shortly to have an Honours degree (2i ir better) in a Biological Science.

Applications, with curriculum vitae and names and addresses of two referees, should be sent to Professor H W Woolhouse at the above address, not later than 20 June 1984.

(2290)F

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF PLANT BIOLOGY

RESEARCH STUDENTSHIPS

Applications are invited for the following studentships tenable from October 1st.

1. SERC CASE Studentship in collaboration with MAFF Laboratory in Harpenden, on the detection and elimination of viruses in true potato seed.

2. SERC CASE Studentship in collaboration with the Scottish Crops Research Institute, on physiological aspects of frost tolerance in blackcurrants.

3. SERC Quota Studentship, tenable in one of the following subject areas: biochemical aspects of plant-pathogen recognition; physiology of aluminium tolerance in tree mycorrhizas; physiology and ultrastructure of chalk and silica deposition by algae.

Further details are available from Professor J A Callow, Department of Plant Biology, University of Birmingham, B15 2TT and applications should contain a full cv and names of 2 referees. (2261)F

UNIVERSITY OF LANCASTER

DEPARTMENT OF ENVIRONMENTAL SCIENCES

SERC RESEARCH STUDENTSHIP

Tenable from 1st October, 1984

Applicants holding or expecting at least an upper second class honours degree in a physical/geophysical science field may apply for either of the following topics:

1. Study of fluid dynamic processes in silicate magma/sulphur systems with application to volcanism on Io.

2. Investigation of the photometric properties of the surfaces of Ganymede, Callisto and Europa using Voyager data.

Applicants should send cv and names and addresses of two referees to Dr L. Wilson, Dept. Environmental Sciences, University of Lancaster, from whom further information can be obtained. **(2296)F**

WYE COLLEGE (University of London)

MAFF STUDENTSHIP IN AGRICULTURAL LAND CLASSIFICATION

Tenable for three years, starting on October 1st, the studentship is to relate crop yield and economic performance to physical land properties. Applicants, preferably competent in the use of computers, should hold or expect to obtain a First or Upper Second Class Degree. Apply, with Curriculum Vitae and the names of two referees, to Dr C P Burnham, Department of Environmental Studies and Countryside Planning, Wye College, Ashford, Kent. TN25 5AH. Tel No (0233) 812401.

Wye College is an equal opportunities Employer. (2281)F

UNIVERSITY OF LEICESTER

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

SERC RESEARCH STUDENTSHIP IN PHARMACOLOGY

Applications are invited from good honours graduates, or from those expecting to graduate in pharmacology, biochemistry or physiology this year, to work on the following project from October 1984.

Interactions of neurotransmitter receptors linked to adenylate cyclase and inositol phospholipid hydrolysis in brain tissue.

The study will involve techniques to directly examine inositol phospholipid hydrolysis, cyclic nucleotide production, receptor labelling and assay of endogenous neurotransmitter release from brain slices.

Applications (including cv and the names of two referees) should be sent to Dr S R Nahorski, Department of Pharmacology and Therapeutics, Medical Sciences Building, University of Leicester, University Road, Leicester, LE1 7RH, as soon as possible. (2241)F

UNIVERSITY OF EDINBURGH

DEPARTMENT OF BACTERIOLOGY

MRC RESEARCH STUDENTSHIPS

Applications are invited for one of the following projects.

1. Phagocyte lectin receptors and their role in immunity to bacteria.

2. The pathogenesis and cell surfaces of Bacteroides species.

3. Detection of Hepatitis B by DNA cloning techniques.

Applicants should hold, or expect to obtain, a 1st or upper second class honours degree in a relevant biological science.

Applications, including a curriculum vitae and names of two academic referees should be sent, as soon as possible, but in any case not later than 20 June, to Professor J G Collee, Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG. (2294)F

PORTSMOUTH POLYTECHNIC

Biophysics Group

SERC POSTGRADUATE STUDENTSHIPS IN CHROMATIN RESEARCH

Applications are invited from graduates or final year students in biochemistry, chemistry or a related subject. The projects will involve the study of structure/function relationships in chromatin at the level of the nucleosomal supercoil.

Further particulars and application forms from Professor C Crane-Robinson, Biophysics Laboratories, Portsmouth Polytechnic, St Michaels Building, White Swan Road, Portsmouth. PO1 2DT. Ref: B16.

(2260)F

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

THE COURTAULD INSTITUTE OF BIOCHEMISTRY

London W1P 7PN

CANCER RESEARCH CAMPAIGN STUDENTSHIP

Applications are invited for research studentship within the CR Endocrine Tumour Molecula Biology Group under Professor R Craig for a period of three years fro 1 October 1984. The project will b concerned either with the structu and regulation of the hyma calcitonin/CGRP gene in thyroi and lung tumours, or the applicatio of recombinant DNA techniques i the study of human breast cancer.

Candidates should have or expe to obtain a first or upper second cla honours degree preferably in Bi chemistry or Genetics.

Applications enclosing names an addresses of two referees should b sent as soon as possible to Professo R K Craig at the above address.

(2269)F

STUDENTSHIPS

QUEEN MARY COLLEGE (University of London)

SCHOOL BIOLOGICAL SCIENCES

SERC CASE STUDENTSHIP

THE MOLECULAR GENETICS OF PROTEASE SECRETION IN YEAST

Applications are invited for the above studentship. The successful applicant would be expected to register for a PhD degree. The project in collaboration with Distillers Company (Yeast) Ltd, will be jointly supervised by Dr I Evans (DCL) and Professor E A Bevan (QMC).

Applications with a curriculum vitae and the names of two referees should be sent as soon as possible to Professor E A Bevan, School of Biological Sciences, Queen Mary College, Mile End Road, London, E1 4NS.

The School of Biological Sciences has three SERC studentships available for work on a wide range of biological topics. Further details available from the above address. (2271)F

UNIVERSITY OF EDINBURGH

DEPARTMENT OF VETERINARY PHYSIOLOGY

SERC RESEARCH STUDENTSHIP

Applications are invited for a studentship tenable for three years from October 1984 for studies leading to the PhD degree. Projects available are in the area of peripheral and central somatosensory neurophysiology.

Enquiries to: Dr A D Short, Department of Veterinary Physiology, Royal (Dick) School of Veterinary Studies, Summerhall, Edinburgh EH9 1QH. Tel: 031-667 1011 ext 5249. (2265)F

UNIVERSITY OF NOTTINGHAM

DEPARTMENTS OF BOTANY AND PHARMACY

POSTGRADUATE RESEARCH STUDENTSHIP IN MICROBIAL BIOCHEMISTRY

Applications are invited for a studentship funded by a University of Nottingham Research grant. The project involves a study of the physiology and biochemistry of production of antifungal antibiotics under the supervision of Dr J F Peberdy (Department of Botany) and Professor B W Bycroft (Department of Pharmacy).

Applicants should have or expect to have a First or Upper Second Class honours degree in Microbiology and/or Biochemistry. The award is for two years, in the first instance, commencing October 1984.

Applications, including the names of two academic referees should be sent to Dr J F Peberdy, Department of Botany, University of Nottingham, Nottingham NG7 2RD. (Tel 0602 506101 ext 2204) to arrive not later than June 22nd, 1984. (2287)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

TWO RESEARCH STUDENTSHIPS

Two MRC funded Research Studentships are available for work on the following projects:-

(a) The retrograde axonal transport of proteins in peripheral nerves

(b) The kinetics and pharmacological effects of primaquine formulations

The latter project will be carried out in association with the Department of Parasitology, Liverpool School of Tropical Medicine.

Candidates should possess or expect to obtain a first or upper second class honours degree in pharmacology or related subjects.

Applications to the Registrar, The University, PO Box 147, Liverpool L69 3BX. Quote Ref: RV/530/N. (2232)F

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF PURE AND APPLIED BIOLOGY

SERC RESEARCH STUDENTSHIP

Applications are invited for a studentship starting 1 October 1984 for three years. The work involves the experimental and/or theoretical study of population dynamics in heterogeneous environments using insects as subjects.

Applicants must possess or expect a good honours degree in biology of mathematics and should send a full CV and arrange for two references to be sent to Professor M P Hassell, Imperial College, Silwood Park, Ascot, Berks SL5 7PY. (2293)F

UNIVERSITY OF LONDON

INSTITUTE OF PSYCHIATRY

DEPARTMENT OF NEUROLOGY

SERC RESEARCH STUDENTSHIP

Applications are invited for a CASE Studentship tenable from October 1984. The position is renewable for 3 years subject to satisfactory progress. The applicant will be expected to register for a higher degree.

The work involves the behavioural assessment of neuroleptic-induced abnormal involuntary movements in rodents. The collaborating body is Organon Laboratories.

Applicants should be recent graduates in a biological science with a first class or upper second class honours degree.

Applications, with a curriculum vitae and the names of two references, should be sent to Dr P Jenner, Department of Neurology, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF. Closing date: 29 June 1984. (2270)F

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London)

DEPARTMENT OF NEUROLOGICAL SCIENCE

ANTIGENIC DETERMINANTS ON HUMAN ACETYLCHOLINE RECEPTOR

An MRC Studentship

is available for 3 years from October 1984 for a Research Student to join an MRC Programme Grant-funded group of basic and clinical scientists studying the cause and treatment of myasthenia gravis, to work on the antigenic determinants on human acetylcholine receptor using biochemical techniques and monoclonal antibodies.

Applicants should have or expect to obtain a first class or upper second class degree in biochemistry or immunology.

Curriculum Vitae, including the names of two academic referees, should be sent to Dr A Vincent, Department of Neurological Science, RFHSM, Rowland Hill Street, London NW3 2PF by 22 June 1984. Further details are available by request. (2286)F

THE UNIVERSITY OF SHEFFIELD

POSTGRADUATE STUDENTSHIPS IN TUMOUR BIOLOGY

Applications are invited for 2-3 studentships in the Departments of Surgery and Virology tenable from 1 October 1984. The successful candidates will work in the field of tumour biology/biochemistry interface.

Applications, including curriculum vitae and the names of two referees, should be sent as soon as possible to Mr K Rogers, Clinical Sciences Centre, Northern General Hospital, Sheffield S5 7AU. Please quote ref R96/G. (2254)F

CHARING CROSS HOSPITAL MEDICAL SCHOOL (University of London)

DEPARTMENT OF BIOCHEMISTRY

SERC Post Graduate Studentship

Applications are invited for a three-year studentship leading to a PhD.

The candidate will work in the newly established Gene Structure and Function Group, either with Dr L A Archard or with Professor A D B Malcolm. Projects include: Analysis of Coxsackie virus; Orthopox virus gene expression; Campylobacter toxin gene expression; Effects of DNA conformational changes; Role of nucleosome phasing in transcription.

Further details are available by telephoning 01-748 2040, ext 2835. (2259)F

UNIVERSITY OF WARWICK

POSTGRADUATE STUDENTSHIPS IN BIOLOGICAL SCIENCES

Applications are invited from persons possessing, or expecting to obtain in June 1984, a First Class or Upper Second Class Honours Degree in Microbiology, Biochemistry, Genetics or a related subject to work on the following projects:

i) An SERC Biotechnology Directorate-funded project to work on the biochemistry and physiology of thermophilic aromatic-utilizing bacteria with a view to considering their potential application in the production of high value added chemicals. The studentship will be under the supervision of Professor H Dalton.

ii) An SERC Quota funded project to work on the molecular biology on the heterocysts in Cyanobacteria. The studentship will be under the supervision of Professor N G Carr.

Applicants should send a cv, together with the names and addresses of two referees, to the Executive Officer, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, indicating in which of the above studentships they are interested.

Informal enquiries can be made direct to Professor H Dalton at the above address, 0203-24011, ext 6025, or direct to Professor N G Carr, Biochemistry Department, University of Liverpool, 051-709-6022, ext 2518. (2268)F

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF PHYSICS

SERC PhD STUDENTSHIP

AMORPHOUS AND CRYSTALLINE QUANTUM WELL SEMICONDUCTORS

Applications are invited from graduates with a class I or II.1 Honours degree in physics, materials science or electronic engineering, for a SERC Information Technology award, tenable from 1 October 1984 to study photoluminescence in extremely thin layer structures of amorphous silicon which show quantum size effects. Comparative studies of GaAs superlattices will be included. The successful candidate will join an established group in the field of amorphous semiconductors especially interested in the study of photo- and electro-luminescence, using lasers and related opto-electronic techniques. This work is being extended to include quantum size effects in both amorphouse silicon and crystalline III-V semiconductors. The group collaborates actively with a number of workers in the device field. The project will involve a wide range of solid state techniques with emphasis on the physics of these materials.

Applications, with curriculum vitae and the names of two referees to Professor I G Austin/Dr T M Searle, Department of Physics, The University, Sheffield S10 2TN as soon as possible. Please quote ref no R93/G. (2252)F

CONFERENCES and COURSES

UNIVERSITY OF SUSSEX

M.Sc. Courses in Biochemistry

The University offers four full-time, one-year M.Sc. courses in Biochemistry:

GENERAL BIOCHEMISTRY
BIOCHEMICAL ENDOCRINOLOGY
GENETIC MANIPULATION AND MOLECULAR BIOLOGY
PLANT BIOCHEMISTRY AND BIOENERGETICS

and places (without financial support) on most of these are available for the 1983/84 session. General Biochemistry may also be taken as a two-year course. Research projects are included in each course, related to research currently underway in the Biochemistry Group. Applicants should normally possess at least a 2nd Class Honours degree or its equivalent in Biochemistry or in a Biological, Chemical or Medical Science.

Research Studentships in Biochemistry

Several research council studentships are available to support students working towards a research degree. One of these is earmarked to an SERC grant for **immunological studies of plant mitochondrial biogenesis** (Dr A.L. Moore). Others may support students working on any of the research topics of interest to the department, especially: (1) **regulation of transcription in eukaryotes**, (2) **immunoassays for nuclear antigens**, (3) **hormonal and nutritional control of growth and protein synthesis**, (4) **hormonal regulation of gluconeogenesis and ureogenesis.**

Application forms and further details for both MSc. courses and research studentships may be obtained from the Subject Chairman of Biochemistry, School of Biological Sciences, University of Sussex, Falmer, Brighton, Sussex BN1 9QN. (2297)C

THE UNIVERSITY OF LEEDS
MSc IN CARDIOVASCULAR STUDIES

A one-year course which provides a scientific training for investigations into the human and other cardiovascular systems. The course is open to graduates in medicine, physiology and related disciplines and commences 1 October.

For further details write as soon as possible to the Course Tutor, Department of Cardiovascular Studies, The University, Leeds LS2 9JT. (2255)C

Southampton THE UNIVERSITY

MSc in Electrochemical Science

This SERC approved course offers a balanced combination of lectures and practical work. The aim of the course is to introduce the techniques and concepts of electrochemical science and to show how they are applied to practical problems. In the second half of the course students carry out a research project. Assessment is by written examination and by oral examination of a dissertation. SERC studentships are available for suitably qualified applicants.

Further details may be obtained from:
Dr L.M. Peter
Department of Chemistry
The University
Southampton SO9 5NH
(2285)C

GRANTS and SCHOLARSHIPS

WELSH NATIONAL SCHOOL OF MEDICINE
(University of Wales)
DEPARTMENT OF CHILD HEALTH

Applications are invited for a post-graduate scholarship to investigate androgen action and metabolism in disorders of sexual differentiation in humans, using tissue culture and cell fractionation techniques.

Applicants should have, or expect to gain, a first or upper second class honours degree in Biochemistry or a related science discipline.

The scholarship will commence in Summer/Autumn 1984 and is tenable for a 3 year period (subject to a probationary year). The results are expected to be submitted for a PhD degree.

Further particulars and application forms (quoting Ref No C10/62) may be obtained from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN (Tel No 0222-755944 Ext 2296) to whom applications should be sent by 25th June, 1984. (2274)H

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WORKSHOP

WORKSHOP ON ABNORMAL CELLS, NEW PRODUCTS, AND RISK

Co-sponsored by:
FDA, NCI, NIAID, NHLBI, and CDC
will be held on July 30-31, 1984
at the NIH in Bethesda, MD, USA

The meeting has been organized to review issues and data relating to the use of abnormal cells in the production of drugs and biologics. Particular attention will be focused on three areas: (1) residual cellular DNA in products; (2) viruses endogenous to the cell substrate; and (3) residual cellular proteins in products. Discussions will follow the presentation of data, and there will be an attempt to develop consensus in each of the three areas during the afternoon of the second day. The Public Health Service Agencies co-sponsering this workshop believe it will assist them in making decisions on the support of research, the regulation of products, and the use of products derived from abnormal mammalian cells. The meeting is open to the public; however, individuals planning to attend should send their name and address to: Mary Ann Tourault, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20205 so that additional information can be sent in advance of the meeting. (NW575)V

FELLOWSHIPS *Continued from p. 16*

DEEP DIVE RESEARCH

As part of a research program in deep dive medicine The Norwegian Research Council for Science and the Humanities has established 5 3-years Research Fellowships within neurology/neurophysiology/thermophysiology, circulation and farmacology.
The salary is Nkr 113.996-192.119, depending on qualifications. Further information may be requested. **Application and 3 copies of scientific papers within June 25 1984 to: Norwegian Research Council for Science and the Humanities, Munthes gt. 29, 0260 Oslo 2, Norway. Telephone (02) 56 52 90** (W1034)E

UNIVERSITY OF ABERDEEN
DEPARTMENT OF OPHTHALMOLOGY
POST-DOCTORAL RESEARCH FELLOWSHIP

Applications are invited fro a post-doctoral position to work on the cell biology of proliferative retinal disease. The work will initially be directed towards developing an in vitro model of retinal angiogenesis using isolated retinal capillary endothelial cells grown on vitreous explants and artificial collagen substrata.

This is the first of a number of research appointments to the new University Department of Opthalmology and the successful applicant will be expected to participate in the development of the Department. Applicants should have or should be about to complete a PhD in Moecular and/or Cell Biology.

Salary will be on the Range 1A scale for Research and Analogous Staff £7,190 to £11,615 per annum (under review) with appropriate placing.

The position is available immediately, although the starting date is negotiable, and will be for 3 years.

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 29 June 1984. (2282)E

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF BIOCHEMISTRY

POST DOCTORAL RESEARCH FELLOWSHIP

Applications are invited for the above SERC funded post to work with Dr S J W Busby on a study of the regulation of gene expression in E coli at the level of transcription and translation. In particular the candidate will investigate the role of messenger sequence in the efficient initiation of translation. Applicants should have an interest in the application of recombinant DNA technology.

Post tenable for up to three years from the soonest possible date.

Salary on the Research Fellow 1A scale £7,190 to £11,615 plus superannuation.

For further particulars phone 021 472 1301 ext 2559, quoting reference B6.

No formal application form. Three copies of application, including full curriculum vitae and naming three referees to Assistant Registrar (Science and Engineering), PO Box 363, Birmingham B15 2TT, by 22 June 1984. (2243)E

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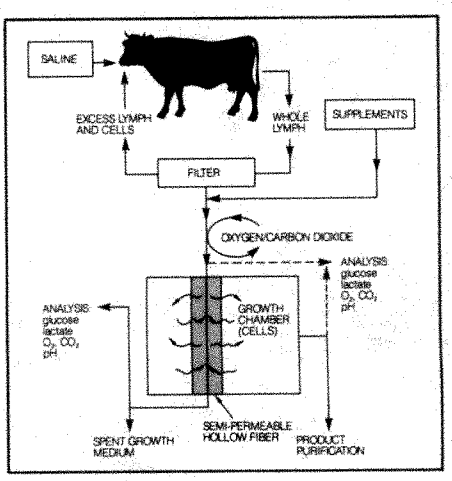
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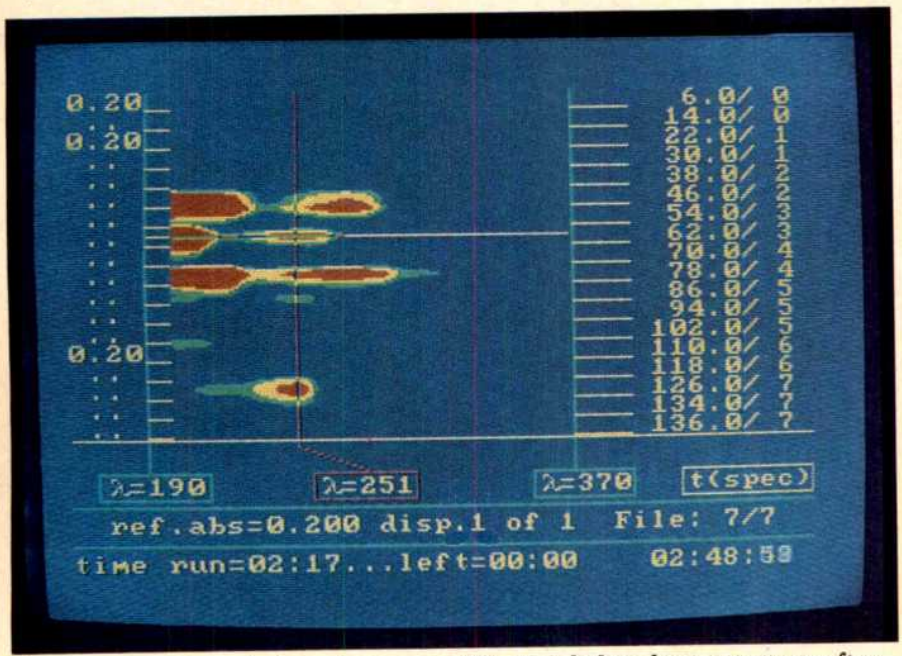
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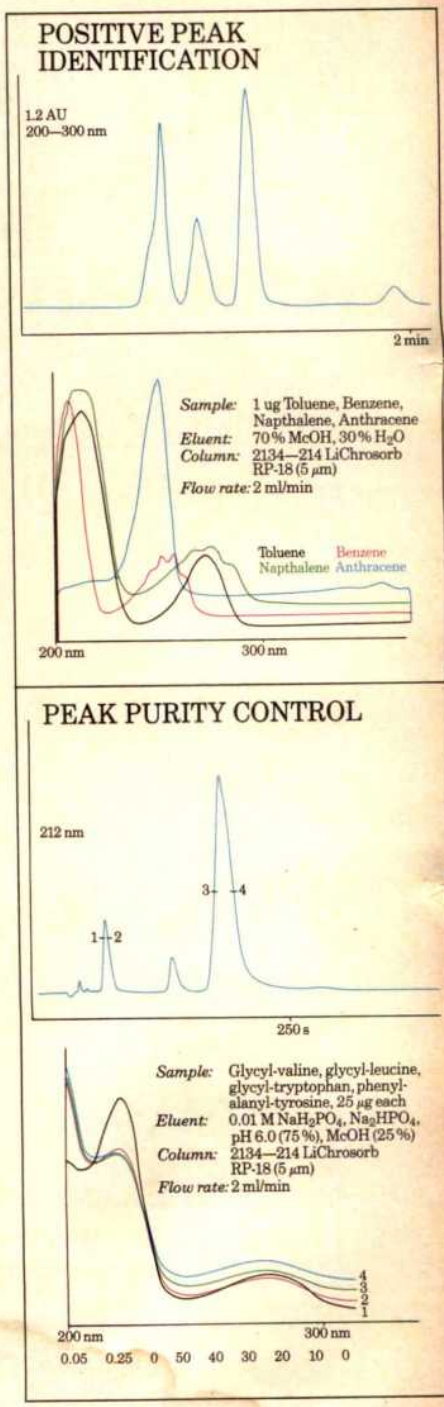
COMPUTERS IN THE LABORATORY

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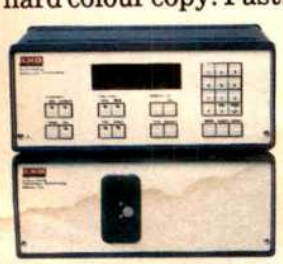
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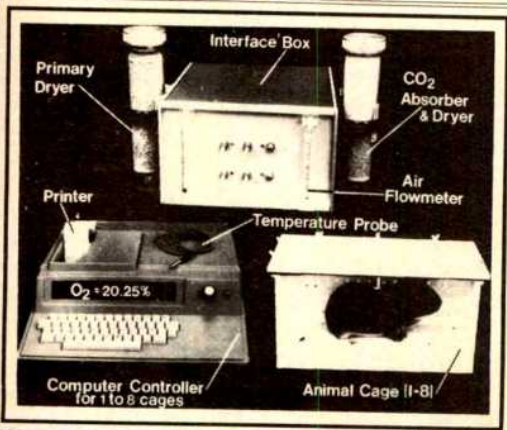
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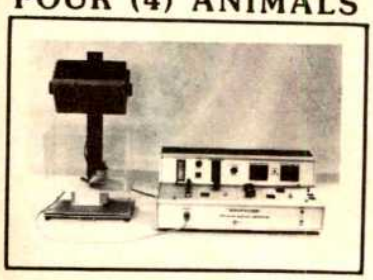
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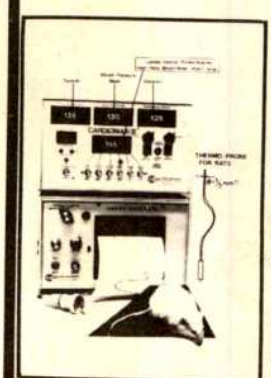
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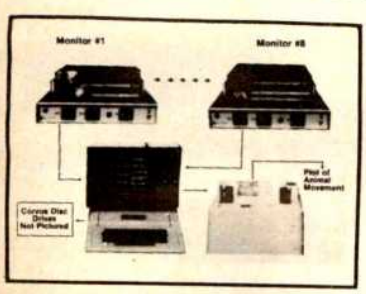


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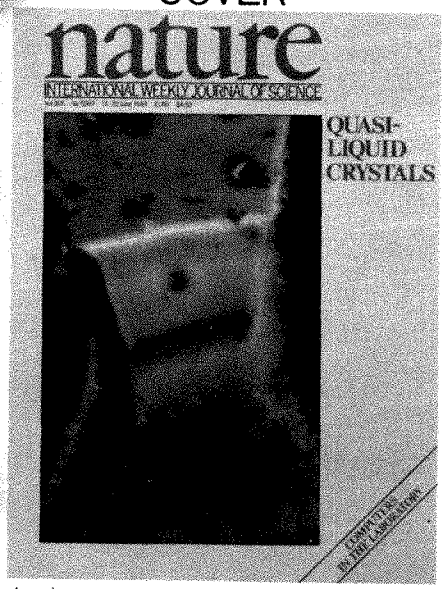
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nature

NATURE VOL. 309 14 JUNE 1984

COVER



A spiropyran quasi-liquid crystal viewed through a polarization microscope. These crystals have the properties of conventional liquid crystals, but also show structures not normally seen in such materials, for example clusters of non-birefringent microcrystals. Quasi-liquid crystals are discussed on p.608.

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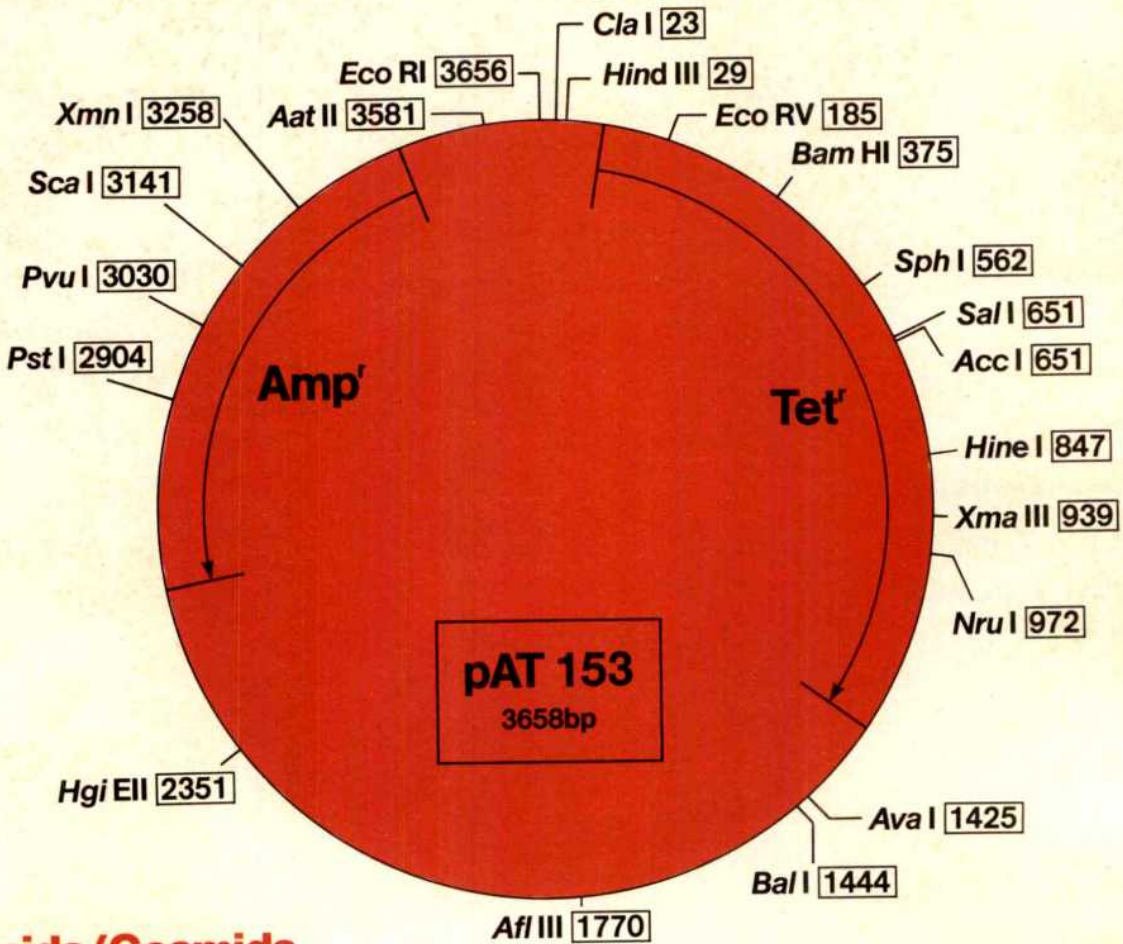
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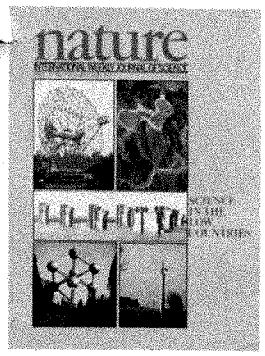
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September 10-12, 1984
Boston Park Plaza Hotel

Daily Themes:
DAY 1 - ONCOGENES,
DAY 2 - THE CANCER CELL,
DAY 3 - PROSPECTS/PROBLEMS.

MONDAY, 10 September
Exhibition Viewing: 8:30am — 6:30pm
Recruitment Center: 9:30am — 6:00pm

DAY 1
ONCOGENES AND GROWTH FACTORS — WHAT THEY ARE AND WHAT THEY DO

9:00 — 9:15	**Chairman**
9:15 — 10:00	Oncogenes *R. Weinberg (MIT)*
10:00 — 10:45	Growth factors *M. Sporn (NIH)*
11.15 — 12.00	HTLV *R. Gallo (NIH)*
12.00 — 12.30	Clinician's view *S. Broder (NIH)*
2.00 — 4.00	Round table with morning speakers
4.30 — 5.30	The cancer cell *J. Cairns (Harvard)*

TUESDAY, 11 September
Exhibition Viewing: 8:30am — 6:30pm
Recruitment Center: 8:30am — 6:00pm

DAY 2
THE CANCER CELL — HEREDITARY CANCER, TUMOR PROMOTERS AND CARCINOGENESIS

9.00 — 9.15	**Chairman**
9.15 — 10.00	Gene regulation *P. Leder (Harvard)*
10.00 — 10.45	Hereditary cancer *R. White (Utah)*
11.15 — 12.00	Carcinogens and promoters *I.B. Weinstein (Columbia)*
12.00 — 12.30	Clinician's view *F. Gilbert (Mt Sinai)*
2.00 — 4.00	Round table with morning speakers
4.30 — 5.30	NCI program

WEDNESDAY, 12 September
Exhibition Viewing: 8:30am — 3:00pm
Recruitment Center: 8:30am — 3:00pm

DAY 3
PROSPECTS AND PROBLEMS — DRUG RESISTANCE, MONOCLONAL ANTIBODIES AND NEW DRUGS

9.00 — 9.15	**Chairman**
9.15 — 10.00	Diagnostics and therapeutics *G. Todaro (Oncogen)*
10.00 — 10.45	Immunotoxins *J. Uhr (Texas)*
11.15 — 12.00	Drug resistance *A. Varshavsky (MIT)*
12.00 — 12.30	Clinician's view *J. Gutterman (Houston)*
2.00 — 4.00	Round table with morning speakers
4.00 — 4.45	Prospects
4.45	Closing remarks

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SEM OPIMISATION (Thursday/Friday)	The main objective of this symposium is "Getting the best out of your SEM". A series of tutorial lectures from acknowledged experts will cover the instrument, its operation, specimen preparation, image recording and image interpretation.
TUTORIALS IN MODERN ASPECTS OF MICROSCOPY (Wednesday)	This Symposium is concerned with microscopy and analysis of solid specimens. The lectures will not only cover SEM methods for the non-expert, but also Quantitative Microscopy, Scanning Optical Microscopy, Acoustic Microscopy, and Atom Probe Analysis.
STATE OF THE ART IN TEM AND STEM (Tuesday)	A series of tutorial lectures will deal with imaging and analysis of thin specimens. Among other things, the symposium is intended to update the knowledge of those not working directly with TEM or STEM.
ANALYSIS IN THE SEM (Friday)	In the sessions on X-ray microanalysis and cryo-techniques a number of the presentations specifically deal with preparation of specimens, including by low temperature methods, and their analysis in SEMs.
IMAGE RECORDING (Wednesday)	The symposium will include lectures on optimisation of scanning images, electronic processing of images, and photographic recording of images.
APPLICATION OF SEM (Throughout MICRO)	Throughout MICRO, many of the sessions will include lectures on applications of the SEM to structural observations and microanalysis. Topics including an SEM component include: interfaces in semi-conductors; automated SEM in environmental studies; automatic SEM particle analysis in forensic science; SEM in the study of ancient materials.
EXHIBITION (Throughout MICRO)	It is expected that more than 50 companies will be exhibiting their latest microscopes and ancillary equipment, covering the complete spectrum of instruments and techniques including, of course, full representation of SEM equipment.

Details of the MICRO 84 programme, and further information, are available from:-

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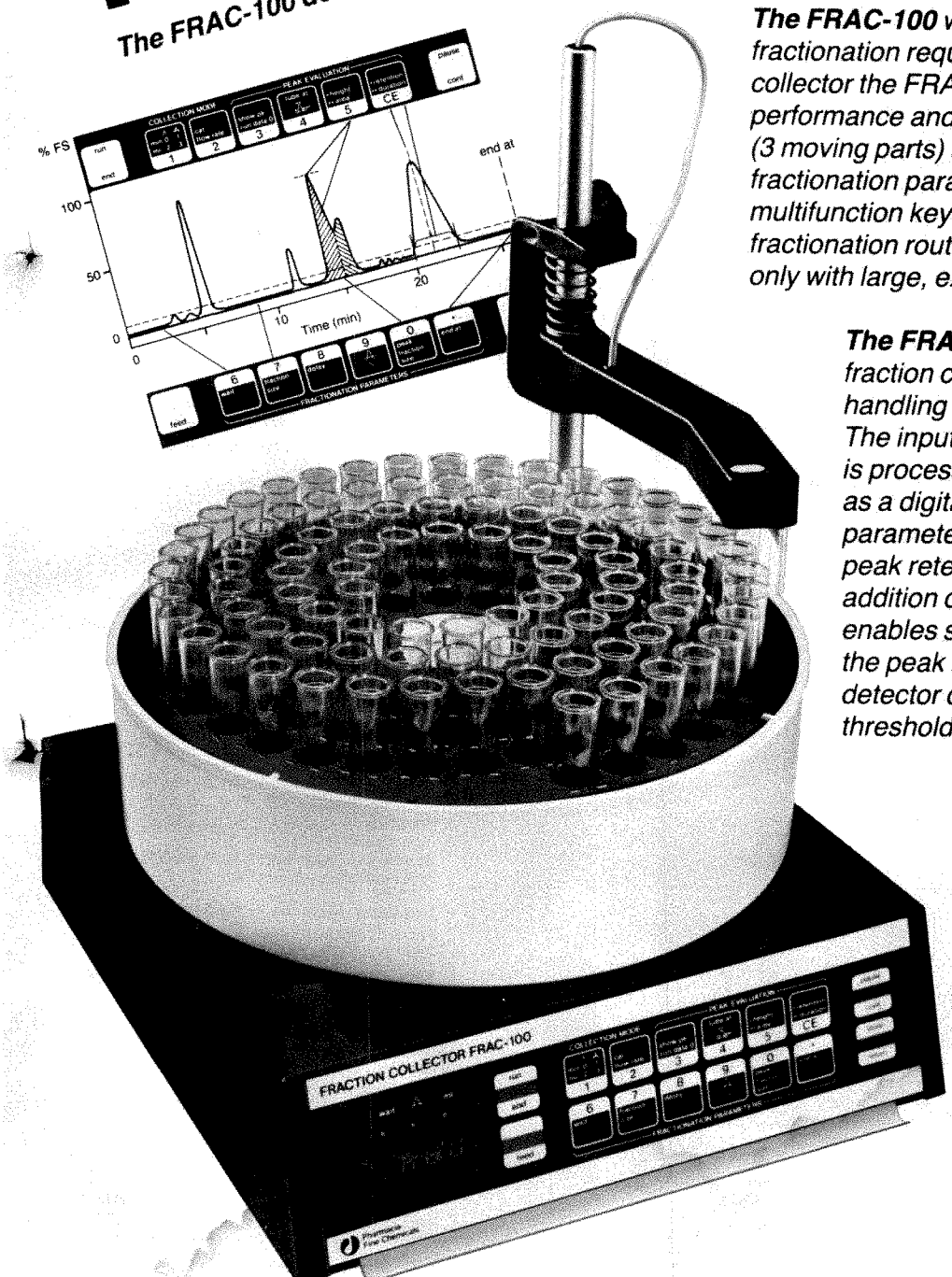
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nature

Planning for next year's summit

Last weekend's London summit was not pointless. And the working group on high technology should seize the opportunities for building a rational framework for innovation.

THE conventional wisdom is that last week's meeting of the heads of government of the seven principal industrialized nations accomplished nothing, which is inevitable. If 4,000 journalists are sent from all over the world to write about a meeting whose proceedings take place in private, frustration and boredom will ensure that no other conclusion will be reached. The nine previous summit meetings were in this respect no different from last week's in London. If those involved are concerned that they are so persistently misunderstood, they have one simple remedy — to hold their private meetings privately, without saying in advance where or when. Then, people's expectations would not be magnified to the point at which they can only be disappointed.

On this occasion, for what it is worth, three substantial benefits have accrued in the eyes of those began by expecting nothing, the chief of which is what the meeting had to say about the management of international debt. The issue is neither arcane nor academic. Increasingly during the past two years, it has become plain that the economic health of all the industrialized nations would be seriously if temporarily damaged if a substantial part of the international monetary system should collapse under the present strain. But why should it matter to people in comfortable industrialized countries, whose chief interest may be the pursuit of some interesting line of enquiry in research, that some distant government should be bankrupt? Surely only a few commercial banks will suffer? That is only half the truth. The Western commercial banks, profit-making and privately held enterprises though they may be, are an essential part of the economic system. And they fall like dominoes, as the events of the 1930s showed all too clearly. The default of a government on its loans, or the collapse of part of the banking system for that reason or some other, would be tantamount to a deflationary impulse within the economy of the industrialized West that would certainly snuff out the fitful economic recovery and might well be worse. The pursuit of research objectives of all kinds would be impeded.

Gloom

Last week's summit acknowledged the truth of this gloomy outlook, which is a good sign. The final communique goes one step further, and says explicitly that the seven major governments will use their influence with their commercial banks to help other governments that "are themselves making successful efforts to improve their position" to pay their debts, chiefly by giving them more time to pay. This is a step forward, but a small step. Better would have been a firm declaration on the size of the United States deficit, which is a cause of high international interest rates because it is not matched by private domestic saving. (Japanese governments can safely run huge deficits because thrifty Japanese, saving for their retirement, forgo current consumption to help out their government.) The statement earlier this week by the new government of Argentina that it had not so far agreed with the International Monetary Fund on a programme of austerity, which is how the summit governments would have it "take steps" to improve its position, suggests in any case that what is now proposed will not work. Politically, the industrialized nations cannot let a newly elected government in Latin America go to the wall, but the commercial banking system could not absorb the debts which it has piled up. On this crucial issue, the summit accomplished something, but probably too little.

On East-West relations — and arms control particularly — the summit declaration is more encouraging. What seems to have been agreed is that the seven members differ in their eagerness to resume negotiations of some kind with the Soviet Union — and have been given the assurance of the others that, up to a point, to do so will not count as rank disloyalty. The result, inevitably, is that governments other than that of the United States will be trying their luck in Moscow in three months ahead. President François Mitterrand, indeed, announced immediately after the summit meeting that he will be off late in the month. By coincidence, the Pugwash organization came to the conclusion, at its Geneva meeting at the beginning of the month, that this was precisely a period when explorations of what might be done on the limitation of nuclear arms would be more productive than formal negotiations. Part of the explanation is that the United States will soon be in the thick of an election campaign. But that, by itself, is no reason why President Reagan should not bring forward the Threshold Test-Ban Treaty for ratification (see *Nature* 7 June, p.482).

Innovation

The third respect in which last week's meeting deserves at least lukewarm applause is that a gathering concerned chiefly with the world's economy found time to approve, if only formally, a report from the Working Group on Technology, Growth and Employment (see p.572). The credit for that goes to President Mitterrand who, in the days of of M. Pierre Chevènement, then the French minister for research, startled the Versailles summit two years ago with his passionate advocacy of technological innovation as the engine of the modern economic system — and as the best way of solving more immediate problems. And while the habit of listening politely to what the working group has to say may be a pale shadow of the Mitterrand evangelism of two years ago, by accident the summit members seem to have created the essence of an institution that could yet render invaluable service to them and the rest of us. Much will depend on what happens in the months ahead.

The working group, although a creature of the member governments, is mercifully above the political battles in which most of them are habitually engaged. No doubt that will remain the case so long as the group is not drawn into arguments about the rights and wrongs of monetarist as distinct from Keynesian economics, or other contentious matters of that kind. Its first instincts, exercised in the preparations for last year's summit at Williamsburg, were to encourage member governments to take the lead in collaborative technical projects (eighteeen altogether).

Gingerly, it has now gone so far as to refer to some of the ways in which the governments which are collectively its master make things needlessly difficult for themselves by their imposition of restraints on technology. This year's topics are the restraints on the free flow of information ordained by strategic considerations (chiefly in the United States) and the tendency of all governments to believe that they too can excel at everything provided they deny themselves and their industries the benefits of technologies developed elsewhere, sometimes by tariffs, sometimes by other means. While the working group's report does little more than define these problems, is has in the process won for itself a chance to contribute to their solution. ▶

This is why it is not too soon to begin preparing the group's report for next year's summit. The most urgent need is that governments should be made to understand the benefits of genuinely free trade in technology, a principle too often illegally denied even within the framework of organizations such as the European Community. Directly or indirectly, all the governments represented in London last weekend are in the business of supporting technological innovation, always by some device for making sure that public purchasing of high-technology products should benefit domestic producers. The immediate consequence is always to postpone a true division of labour in high-technology products, but in the long run, as water finds its own level, it is discovered that it is best, and most economical, for people to manufacture only those parts of products which they can make most economically. Meanwhile, for as long as this truth can be concealed, everybody loses.

The psychological trick that the working group should aim to engineer in preparation for next year's summit is that of persuading member governments that not all of them can be the world's sole supplier of, say, telecommunications equipment or of biologically engineered pharmaceuticals, but that these (and other) technologies are promising enough to allow everybody a sizeable share of a huge cake. That task will not be accomplished by rhetoric alone, for which reason there is an urgent need that the working group's next report should be soundly based in careful analysis and research. In passing, the working group might earn itself some extra credit by securing some practical recommendations on how to save effort in high-energy physics or planetary exploration (see p.573) by sensible collaboration between governments. The group's existence may be fortuitous, but the opportunities in its grasp would not otherwise easily be created. Let us hope it seizes them. □

Postscripts on India

The letters on Indian science on p.578 are the first of a flood. They deserve a careful reading.

WHEN the issue of *Nature* concerned with the condition of science in India appeared on 12 April, the troubles in the Punjab had been rumbling away for at least a year. They have since, as the world knows, exploded at Amritsar, and everybody's concern is that the largest true democracy ever will survive intact. Indians are always rightly saying that many of their apparently built-in obstacles to efficiency and effectiveness derive from their seemingly perverse attachment to democratic ways. The view that the maintenance of that condition of civility is more important than efficient public administration cannot easily be denied, although it is remarkable and courageous that India, with all its social problems, should have kept to that ideal.

The comments which now appear on the survey of the condition of Indian science (and earlier correspondence on the subject) should be read against this background. Some of the more obvious features of the Indian way of running academic and scientific institutions, open access at the universities for example, are largely consequences of the attachment to democracy. (And there is no reason why a state with more than 700 million people should not have more than 140 universities.) But when times are peaceful, there are good reasons why attention should more seriously be paid to the principles of good administration in these institutions. Laboratory directors and vice-chancellors need more freedom to direct their institutions; their colleagues and their students need more tangible goals on which to pin their ambitions. The huge scientific community could itself do much to improve the standards to which the majority work — and there are signs that such a movement is under way.

The question why India needs a scientific enterprise on such a scale, when there is all that poverty (and now Amritsar) needs only the simplest of answers. It has paid off, and handsomely. What is called the Green Revolution is a direct consequence of the investment there has been in India in agricultural research over the past few decades. □

Solar System unknown

A modest case for collaborative exploration of the Solar System deserves cautious support.

THE intended planetary explorers of the United States and Europe are well on the way to breaking records for advance planning (see p.573). Their proposals for a series of collaborative projects would, if accepted, put a cap on planetary exploration between now and the end of the century, at least so far as the industrialized West is concerned. (The Soviet Union and Japan, and perhaps other entrants to this expensive way of gathering data about the Solar System, may yet have surprises up their sleeves.) By comparison, even those who build accelerators are fleet of foot, counting themselves unlucky if they spend more than six years in building a new, more powerful machine. The reason why planetary projects are so long in gestation is that each of them is based on a purpose-built space vehicle packed with an array of instruments, most of which must be specially designed and developed afresh for each intended mission. Costs are also high — $500 million seems to be the standard planning unit. So the question inevitably arises whether there is not some simpler and cheaper way of gathering the same kinds of data. Precisely that question has prompted the US National Aeronautics and Space Administration (NASA) to design a series of modular spacecraft for future planetary exploration.

Within the terms of reference of the joint US–European study, the report now published is nevertheless as sensible as could be expected. Its hallmark is indeed its modesty. One of the assumptions is that the programmes of planetary exploration planned separately by NASA and by the European Space Agency (ESA) will continue, and that the objective of collaboration between the two continents should be to mount projects that are unlikely otherwise to be carried out, yet which together do not carry spending on either side of the Atlantic above the limits likely to be found tolerable. Plaintively, the report says at one point that it would be easy to add to the list of explorations now proposed (to Saturn, six asteroids and Mars) if only there were funds available. The sad truth is that there is unlikely to be unforeseen growth in the budgets, and that the US commitment to the development of a space station (noncommittally endorsed by last week's summit meeting) is likely to take up any slack that may emerge. For the time being, at least, planetary exploration will be a poor cousin to the other projects in the two agencies' portfolios.

While the cost of these space missions is necessarily high, the more serious difficulty for those who would see more spending in this field is conceptual. Once a decision has been made to launch a spacecraft towards, say, Saturn, it becomes impossible not to pack the vehicle with a whole array of instruments devised to glean information about several unrelated phenomena. The result is that these enterprises take on the character of expeditions to distant places of the kind mounted by terrestrial explorers in the nineteenth century. Two of the projects now suggested (to the asteroids and to Mars) have the further disadvantage that they are meant as spurs to the development of new technology (solar-electric propulsion and robotized exploration), further clouding the objectives. It would, however, be a shame if European planetologists have to twiddle their thumbs for the next decade and a half, brooding over the meagre harvest of data ESA plans to provide. If the worst comes to the worst, at least the Saturn project should be mounted.

Meanwhile, planetary explorers should bend their minds to two other tasks, one technical and one conceptual. The case for planetary exploration would carry more conviction if space probes were simpler, cheaper but also faster — having to plan now to make observations more than a decade hence is to be bound to an inflexible chariot-wheel. And it would also strengthen the planetologists' case if they were to list the questions about the Solar System that need most urgently to be answered and then to specify the data that most directly bear on them. Part of the trouble now is that the questions they are asking are not pointed enough to justify spending in lumps of $500 million. □

UK higher education

Cheers but no extra funds for engineering

THE British Government's ambition to redirect higher education towards engineering and physical science has been at least temporarily set back. At a meeting last week (4 June) called by Sir Keith Joseph, Secretary of State for Education and Science, it became plain that other spending ministries were not prepared to contribute by a reduction of their own budgets towards the cost of what the government is proposing.

There is particular resentment among officials of the Department of Education and Science that the Department of Trade and Industry, the chief agitator within the government for an increased output of graduates in engineering and physical science from British universities and polytechnics, was in the event unwilling to back its rhetoric by an agreement that funds should be diverted from its own budget for a new investment in higher education.

Last week, Sir Keith Joseph was asking for an extra £400 million to be spent over the next several years in universities and polytechnics, chiefly on new buildings and equipment for engineering and physical science departments. The government appears to have been convinced that such a development is urgently necessary by the well publicized complaints by engineering companies that they are having to recruit high-technology specialists from overseas and by evidence from within the educational system that the provision of buildings and equipment has for several years fallen short of needs.

In Whitehall, the plan to redirect resources towards engineering and physical science education has for several months been known as "The Switch", in retrospect a galling echo of the title of the 1970s film about confidence trickery, "The Sting". The objective has been to engineer a decisive and, with luck, permanent shift in British higher education towards the preparation of students for work in high-technology industry. The estimated cost of £400 million is predicated on the assumption that it would be better to strengthen and enlarge departments which are already strong (and fully stretched) than to take up unused capacity at other institutions.

Even so, one of the surprises of last week's meeting (attended both by Mr Norman Tebbit, Secretary of State at the Department of Trade and Industry, and by Mr Nicholas Ridley, Financial Secretary to the Treasury, the man who administers public spending) is that the estimated cost of £400 million seems not to have been questioned. It is not at this stage clear whether the extra investment in engineering education would have been accompanied by an increase of student numbers at British universities.

While the meeting seems unanimously to have denied Sir Keith contributions towards his £400 million, the spending ministries represented at the meeting seem to have been less niggardly with their promise of wholehearted support for what he is trying to accomplish. But his direct request to the Treasury for the extra funds seems to have been met with the familiar statement that "further study" would be necessary.

With time unexpectedly on their hands, Sir Keith and Mr Tebbit appear to have fallen into a desultory and even nostalgic conversation about the advantages there would be if only UK students were able to finance their own residence at universities. Schemes for replacing government maintenance grants by student loans, fashionable at the beginning of the present government's spell of office, have long since been abandoned because they offer no immediate relief to the public treasury.

The chance that the Treasury may find part of the funds needed to engineer The Switch in time for the financial year beginning in April 1985 is nevertheless not identical with zero. But many educationists are relieved that the plan has for the time being been derailed, if only because British higher education institutions are still in the thick of adjusting to straitened budgets — and bracing themselves for the probable erosion of the boundary between universities and ploytechnics. Even so, in the aftermath of Sir Keith Joseph's failure with his begging-bowl, the University Grants Committee is likely to be devising a system of carrots and sticks to persuade universities towards engineering education. □

The year 2000

Prophets of doom rejected

Washington

A BLISTERING riposte to the pessimistic forecasts contained in the Carter Administration's 1980 *Global 2000 Report to the President*, was unveiled in Washington last week by the Heritage Foundation. *The Resourceful Earth*, a new book coedited by the late Herman Kahn and a Heritage Foundation economist, Julian Simon, says the *Global 2000* study is full of factual errors and illogical and misleading arguments. The book says the *Global 2000* warning that by the year 2000 the world will be more crowded, more polluted and less stable ecologically is "dead wrong". Its own happy prognosis is that the world in 2000 will be less crowded (though more populated), less polluted and more stable ecologically.

The Resourceful Earth concedes that the Earth's population will grow by the year 2000, but argues that better housing and mobility will make life less crowded for most people. In the richer countries, it says, there is evidence that hazardous air pollution has been declining and water quality improving. In rich and poor countries alike, life expectancy is increasing and average income has risen. Acid rain, the book says, is troubling, but could be mitigated by a greater reliance on nuclear energy. And the prospect of running out of energy, given the availability of nuclear power, is "purely a bogeyman".

The body of *The Resourceful Earth* consists of a score of papers examining trends in population, agriculture, climatic trends, energy, health and the environment. According to Simon, many of the authors were incensed by the "bad science" of *Global 2000* and were determined to put the record straight. Although almost all the papers are robustly optimistic, the introduction (which appears under the names of Simon and Kahn although Kahn apparently died before he was able to make a significant contribution), warns against complacency. It concedes that all is not well everywhere and that a better future will not happen automatically or without effort.

Why the stark differences between *Global 2000* and *The Resourceful Earth*? Mainly, says Simon, because the report to the President failed to use enough trend data and, when it did, used them badly. Thus *Global 2000*, analysing data up to 1975, predicted continuing stagnation in the size of the fish catch. *The Resourceful Earth* claims that data for the years since 1975 show the fish catch resuming its long-run upward trend.

If there is one thread of pessimism in *The Resourceful Earth* it has to do with people rather than nature. Several papers complain that science and ingenuity offer ready solutions to many of our problems but humans and their institutions have a habit of botching them. A persistent example offered in the book is the reluctance of Americans to embrace nuclear power. In a dissenting statement at the end of the book, one author, University of Pittsburgh physicist Bernard Cohen, says he cannot share the optimism of his fellow authors because science has come under "irrational attack by the forces of ignorance". Public fear of nuclear power, he argues, is the fault of the media, "a group of scientific illiterates drunk with power, heavily influenced by irrelevant political ideologies".

Peter David

The Resourceful Earth: A Response to Global 2000 will be published in July by Basil Blackwell.

British polygraphology

Getting into business

THE polygraph or lie detector has arrived in Britain. A recently established company, Polygraph Security Services Ltd, now offers polygraph tests as a means of combating employee theft, and boasts that in at least one case now pending, charges of theft have been brought after "identification" of a suspect with the polygraph. In another instance, a man who failed a polygraph test was later found to have previous undeclared convictions for theft and was consequently dismissed.

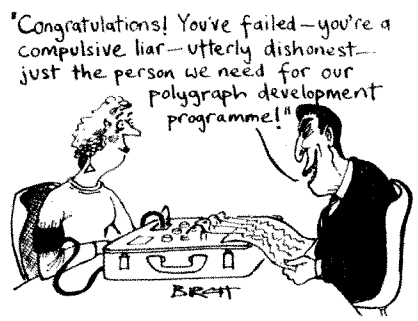
Directors of Polygraph Security Services recently appeared before the House of Commons Select Committee on Employment, which is conducting an inquiry into the implications of the polygraph for industrial relations. The company declined to identify its clients to the Commons committee, citing commercial confidentiality. The committee accordingly threatened to use its legal powers to obtain such information, and the company's directors are now taking legal advice.

The chairman of Polygraph Security Services is Sir George Terry, a former chief constable of Sussex police. The other directors are Mr Jeremy Barrett, Mr Philip Tite and Mr Martin Seligson. Tite and Seligson are directors of Triangle PD Ltd, a printing and publicity company, and Seligson is also a director of Beneficial Arts Ltd, a company which distributes films of horse races to social clubs for fund-raising events. Barrett is director of a market consultancy company. None of the directors has previous experience of polygraph tests. Their training has been through the Zonn Corporation in the United States, of which Polygraph Security Services describes itself as the United Kingdom extension.

Seligson claims that with the polygraph, companies can cut employee theft by 80 per cent. Some client companies are already conducting tests on job applicants, and an existing workforce can be screened regularly through the "honesty maintenance programme" offered by Polygraph Security Services. Seligson stresses that all tests are voluntary, although it is unclear how such a screening programme could possibly be effective if a substantial proportion of the target group declines to be tested.

Seligson is unimpressed by the serious questions that have been raised about the reliability and efficacy of the polygraph. He says that Professor David Lykken, who wrote a critical article on polygraphic interrogation published in *Nature* in February (**307**, 681; 1984) is "totally debunked" and "generally discredited". The authority for this judgement is a paper by D.C. Raskin and J.A. Podlesny published in the *Psychological Bulletin* in 1979 (**86**, 54–59). The empirical issues separating those authors from Lykken boil down to what sort of data is valid for an objective test of the polygraph's effectiveness. Lykken charges that Raskin relies on studies using pre-selected polygraph traces which had previously been scored "correctly" by a polygraph examiner, so providing evidence only that trained polygraphers tend to concur in their judgements.

UK polygraphers are not above circulating (non-attributably) rumours to the effect that Lykken administered or undertook to administer a polygraph test to Mr John de Lorean, now on trial in California, on behalf of de Lorean's defence lawyers. Lykken's version of this story is that the judge in de Lorean's case threw out all polygraph evidence after seeing two directly contradictory polygraph reports: one, from the Federal



Bureau of Investigation, concluded that the accused was deceptive, while the other by Raskin concluded that de Lorean was truthful with 99 per cent probability. Lykken denies ever giving polygraph tests himself except as demonstrations, and says his total involvement with the case was one telephone call.

While Polygraph Security Services is already advertising its services in Britain, little is known about plans to introduce polygraph testing for those employees at the Government Communications Headquarters in Cheltenham who have access to highly classified material. The British Security Commission recommended last year, after a review of the Prime spy scandal, that polygraph testing should be considered for such employees. It is thought that about 20 senior Cheltenham personnel volunteered to take part in a pre-test of the polygraph in London, although some others may have declined. There are no longer any formal links between the majority of employees at the communications headquarters and the civil service unions, but there are not thought to be any immediate plans to extend the test. But, according to one account, it has already been decided in principle to make polygraph tests mandatory for all employees at Cheltenham during their probationary period, and for those with access to highly classified material at each quinquennial security review.

Tim Beardsley

Summit science

Leaders espouse collaboration

THE London summit last week gave an uncontentious blessing to a report of the Working Group on Technology, Growth and Employment — the gathering of senior government science advisers which owes its existence to President François Mitterrand's initiative at the Versailles summit two years ago. But the secret of the report's easy passage seems to have been that its references to contentious issues were cast in the most anodyne language.

On the perennial bone of contention between the United States and the six other summit countries — the control of Western technology by means of the strategic embargo — the report says only that strategic considerations have persuaded governments to seek to control technology and that, "nevertheless", the exchange of information and the encouragement of trade are necessary for the maintenance of technological innovation.

Dr Robin Nicholson, Chief Scientific Adviser at the UK Cabinet Office and *ex officio* chairman of the working group during this calendar year, explained earlier this week that the immediate objective had been to win the acknowledgement of governments that strategically inspired restraints on the free flow of information and products were a potential impediment to the pace of technological innovation. He said that the issue may be explored in greater detail at the two sessions of the group due to take place before the next summit, next year in West Germany.

Other impediments to innovation such as "non-tariff" barriers to international trade are also referred to in the report, which acknowledges that these often stem from the "wish of countries" to be self-sufficient in high technology. Nicholson is especially keen that the working group should encourage uniform and acceptable standards for the new technologies.

The working group's chief initiative since the Williamsburg summit a year ago seems to have been to urge the need for international cooperation on environmental problems, especially acid rain, radioactive waste disposal, the marine environment, the greenhouse effect and development of power sources free of potentially harmful emissions. The working group rejected the notion that these questions should be added to the list of eighteen projects mounted by the time of Williamsburg. Now the summit proper has asked for a detailed report and action plan by the end of this year.

The projects already begun under the auspices of the summit working group are a mixed bag, ranging from thermonuclear fusion, biotechnology and robotics to an attempt to explain people's resistance to new technology (in which the United

Kingdom volunteered to take the lead). According to Nicholson, the robotics projects, led jointly by France and Japan, were a particularly rich source of collaborative research projects, not merely in production technology but in mining, firefighting and agriculture.

The emergence of the working group as an institutional part of preparations for the annual summits of the industrialized nations is something of a surprise. Nicholson explained this week that its objective is largely to identify areas where collaborative research may be useful, whereupon it will be for individual countries to take the lead in fostering particular collaborative projects among summit members. On his view, some of the areas thus identified are certain to be more fruitful than others, while everybody seems reconciled to the notion that some projects will fail for lack of interest.

Even so, Nicholson concedes, there may be a need that the working group should monitor the fate of the projects which its recommendations stimulate. As things are, proprietary information arising in the course of collaborative research projects would remain the property of the partner paying for the research concerned, while information about the conduct of research projects would not necessarily be passed to all interested parties. During the past year, however, it seems to have been agreed that membership of these partnerships should be extended to countries not among the seven summit-going states, with the proviso that outside membership should be allowed when the interests of the project would thereby be served. □

Planetary exploration

Plans for US–Europe launches

A WORKING party of European and US planetary scientists has made a strong bid for a modest layer of frosting on the cake now promised by their respective agencies, the European Space Agency (ESA) and the US National Aeronautics and Space Administration (NASA). The three projects, originally made public at a meeting organized by the working party at Heidelberg in West Germany at the end of April, were put to a meeting of the Royal Society in London last month. But the working group's hope of explicit backing by last weekend's economic summit has not been borne out.

The essence of the recommendations from the joint working group, led by Eugene H. Levy from the University of Arizona and Hugo Fechtig from the Max-Planck-Institut für Kernphysik at Heidelberg, is a set of three planetary missions designed to be complementary to the programmes supported separately by ESA and NASA, and taking account both of Soviet plans (especially for Halley's comet and on Venus) and those being developed in Japan. The projects are:

- A spacecraft bound for orbit about Saturn but releasing *en route* a probe destined to crash on the surface of Titan, the largest satellite of Saturn, measuring atmospheric and aerosol constituents of the atmosphere of Titan on the way. The object would be launched in 1992.
- Exploration of up to half a dozen asteroids would be attempted by means of a spacecraft directed successively to chosen asteroids, spending about a month in orbit about each of them in turn. The feasibility of this project hangs on the development of a solar-electric propulsion system. Recommended launch date is 1994.
- The most ambitious project is a plan to put an automatic vehicle on the surface of Mars for the collection of geochemical and geological data. Both the landing craft and the vehicle would present considerable technical challenges to those working on the project, for which no launch date has been set except that it should be before the end of the century.

The projects singled out by the working group for priority were originally chosen so as to provide opportunities for a measure of independent collaboration in which respective US and European halves would be developed and built independently. The working group says that the first two projects could be contained within the budgets now foreseen for planetary exploration by NASA ($300 million a year) and by ESA, but that the third, perhaps the joker in the pack, would require that extra funds should be set aside.

The working group report emphasized that the three projects are intended to be complementary to the existing planetary programmes of the two agencies. NASA's future strategy is now tied to the development of a series of modular low-cost spacecraft that would be launched from the shuttle by a Centaur rocket. ESA's programme depends on the use of Ariane. The working group also draws attention to opportunities for closer coordination between the existing plans of the two agencies, the coordinated launching of the US and European Martian satellites due to be launched in 1990.

The working group was established jointly by the European Science Foundation and the Space Sciences Board of the US National Academy of Sciences, each of which has endorsed the recommendations. The test of its success will, however, lie with the responses of the operating agencies, NASA and ESA. The former plans in the next several years to concentrate on the development of low-cost planetary exploration as part of its Observer programme, while European planetologists grumble that they are poor cousins to the astronomers in ESA's forward planning. □

Indian nuclear power

Breeder not so fast

New Delhi

THE Indian Atomic Energy Commission has announced that a tiny reactor using uranium-233 as fuel has become critical at the Bhabha Atomic Research Centre (BARC) at Trombay. This is cheering news at a time when the nuclear power programme is under fire for time and cost overruns in commissioning nuclear reactors, frequent breakdowns and a meagre output of the necessary heavy water despite investment.

The reactor concerned is the only operational reactor in the world fuelled by uranium-233, a man-made fissile isotope of uranium produced by irradiation of thorium in a reactor. The fuel has been produced BARC using a research reactor and reprocessing plant.

For India, the importance of the development lies in its long-term nuclear power programme which is to be based on conversion of thorium to uranium-233. BARC has now shown that this may be feasible. The southern Indian states of Kerala and Orissa have deposits of thorium large enough to sustain atomic power generation for more than a hundred years. Eventually, reactors with uranium-233 fuel may be used as a basis for a fuel cycle which breeds new fissile material.

The tiny reactor Kamini developed at Trombay, uses about 500 grams of uranium-233, and will be commissioned at Kalpakkam research centre near Madras in the next three months. It is estimated that even a test reactor will have to be 100 times bigger than Kamini. Meanwhile, a 15 MW test breeder is nearing completion at Kalpakkam. Indian scientists have developed plutonium-uranium oxide fuel for this reactor. France was to have supplied the fuel, but the deal has fallen through because of what India calls the "prohibitive price" demanded by France.

India's second phase of atomic power development is based on the fast breeder system. Feasibility studies and a design are nearing completion for a 500 MW sodium-cooled reactor to be set up in the next decade. The reactors in the first stage are based on pressurized heavy water and indigenous natural uranium. Only the Tarapur plant is using the enriched uranium now being supplied by France.

India has set a target of 10,000 MW for its nuclear power capacity at the turn of the century, which would meet 10 per cent of the expected total power requirements. Five projects are already in operation, with a nominal capacity of 2,240 MW, and two more under construction will add another 510 MW. But, with a gestation period of as much as 10 years, there are doubts about whether India will meet the ambitious 10,000 MW target. **Sunil Saraf**

US agricultural research

Congress digs in heels

Washington

HOPES for an expanded programme of competitive grants for agriculture have been dashed once again by Representative Jamie Whitten (Democrat, Mississippi), chairman of the powerful House of Representatives Appropriations Committee. Last week, the House went along with Whitten's wishes, approving major cuts that his committee had made in the Reagan Administration's request of $50 million for the programme. The new initiative in biotechnology under the programme was cut from $28.5 million to $10 million. Plant science was cut to half of its present level of $15 million (also the amount requested for next year). Also missing from this year's $34,000 million agriculture budget is $5 million approved last year for graduate fellowships.

Representative George Brown, who has been fighting year after year to increase support for competitive grants above the $17 million level where it has stagnated since its inception in 1978, offered an amendment on the floor of the House to restore $10 million of the cuts to biotechnology. The amendment was rejected on a voice vote. According to Brown's staff, there was little hope that the amendment would be passed ("no-one bucks the chairman"), but Brown believed he had made his point and may have garnered some quiet support that will help when the bill goes to House–Senate conference. The Senate, which has yet to act, is likely to agree with the full administration request.

Supporters of an expanded programme of competitive grants had been optimistic this year. Many longstanding opponents — notably the land-grant colleges, which had tended to view competitive grants as a threat to their assured non-peer-reviewed federal support — have recently changed sides. The association of land-grant colleges produced a report last autumn recommending $70 million for competitive grants for biotechnology and two US Department of Agriculture (USDA) research advisory panels had made similar recommendations. The President's science adviser, Dr George Keyworth, has also lent his personal backing.

During the floor debate, Whitten twice dismissed basic research as "finding answers for answers' sake" and suggested that biotechnology research was "based on hopes" that do not address farmers' immediate needs.

Besides ordering the cuts, Whitten's committee earmarked parts of the competitive grants funds for specific research projects — a development that the programme's supporters say threatens to subvert the principle of investigator-initiated peer-reviewed research. Most of the earmarking clearly reflects the committee's desire to rescue congressional pet projects known as "special grants", scheduled to be phased out this year. USDA has never cared much for special grants, a programme of mandated research that gives the department little flexibility. The special grants programmes in soybean research, acid precipitation and alcohol fuels were transferred in their entirety to the competitive grants plant sciences section, a total of $1.7 million in earmarked funds; two new sections were created in the competitive programme to accommodate $3.5 million in special grants programme on aquaculture, gypsy moths, boll weevil and pine bark beetle; and, apparently for lack of a better place to put it, $250,000 for "sugar cane in Hawaii" was inserted in the biotechnology section. Taking into account the cuts and earmarking, the competitive programme received only an additional $497,000 in unrestricted funds over the current appropriation.

Meanwhile, there are increasing signs that USDA's in-house research arm, the Agricultural Research Service (ARS), is taking biotechnology seriously. Both Beltsville, ARS's chief research centre, and an ARS laboratory in Albany, California, are to become centres for biotechnology. Beltsville officials are speaking of quadrupling research in this area over the coming years. The Albany laboratory will also receive increased support, and collaboration is planned with researchers at the University of California at Davis. Most of the new effort will apparently be supported by a shift of existing funding, since ARS is to receive only a 3 per cent increase in next year's budget.

Stephen Budiansky

Data protection

UK bill improved and approved

THE British Government's Data Protection Bill, substantially modified during its committee and report stages, was finally approved by the House of Commons at its third reading last week. The bill sets out to provide for the first time the statutory right of access for individuals to data that are held about them in machine-readable form, while at the same time preventing unauthorized disclosure of such information. Despite the highly contentious exceptions to these general principles, the official opposition declared itself content with the concessions offered by the government last week and did not oppose the bill, which now returns to the House of Lords. It is expected to gain the Royal Assent within the next month.

One of the most controversial clauses of the bill would have allowed medical data to be passed to outside agencies such as the Inland Revenue or the police by people other than health professionals. A medical inter-professional working group under the chairmanship of Sir Douglas Black has drawn up a code of practice to govern the disclosure of medical information, and after extensive lobbying the government has now accepted that these should in principle apply to computerized health data wherever they occur. A government amendment to the bill will allow the Home Secretary to bring in codes of practice, based on those to be used within the Health Service, wherever these precautions are thought necessary.

Universities and examination boards had been worried that the bill's provisions would allow examination candidates access to their marks even before the examination results were officially published. That anomaly has also been rectified; candidates for examinations will in future be allowed access to their marks only 5 months after the request, or 40 days after the examination results are published, whichever should be sooner. Some university vice-chancellors had, however, hoped that candidates' access would be delayed until 90 days after publication of results, to discourage frivolous challenges to the accuracy of examination data from disgruntled candidates.

Other modifications incorporated during the bill's committee and report stages will allow individuals access to data held about them for the purposes of immigration control, and will allow individuals damaged through inaccurate data the right to compensation for damage and distress. The data protection registrar is to play a more active role than was originally foreseen, promoting codes of practice among data users and pursuing complaints raised by individuals about whom data are held.

Blanket exemption in the bill's earlier version that would have allowed the Home Secretary to exclude any or all government-held data from the bill's disclosure provisions have been considerably curtailed, and small data banks held solely for payroll accounting purposes will not now have to register under the act.

Last week's debate was remarkable for the unaccustomed affability and cordiality displayed between the Home Office minister involved, Mr David Waddington, and the speakers of the official opposition, mainly Mr Robert Kilroy-Silk and Mr Denis Howell. Only Liberal Members of Parliament seemed still determined to score every point they could. It seems that the committee debates on this controversial bill were more than usually productive, and most of its former opponents seem satisfied with the changes that have been made. The bill will allow Britain to ratify the European Convention on Data Protection.

Tim Beardsley

NSF moves

Knapp replaced by Bloch

Washington

DR Edward Knapp, director of the National Science Foundation (NSF) for the past two years, will leave his post at the end of this year to return to Los Alamos. This was announced earlier this week by NSF, which said that Knapp will be succeeded by Erich Bloch, now an IBM vice-president, who has spent all his working life with the company.

Knapp's departure is unexpected. During his spell in Washington, he has secured for NSF some of the most substantial budget increases since the foundation's inception. Over the past two years, the budget has grown by more than 35 per cent while NSF's traditional involvement with science education, virtually eliminated during the early years of the Reagan Administration, has been partially restored.

Knapp has been an energetic and stout defender of the NSF's role in the support of basic science but also a loyal member of the administration. One striking feature of his tenure of his office is that the posts of deputy director and of the three statutory assistant directors at NSF, vacant when he was appointed, have remained unfilled.

Bloch is by background an electrical engineer who, unlike his predecessors, has no doctorate. At IBM, he was responsible for the development of the microprocessors for the IBM 360 series of computers. Since 1968, he has held a number of senior management positions with the company and is at present in charge of technical personnel development.

Bloch was born in Germany in 1925, and became a US citizen in 1959. He is a member of the National Academy of Engineering. The director of NSF is appointed by the US President for a six-year term, which appointment is subject to confirmation by the Senate.

Stephen Budiansky

British nuclear power

BNF bites contamination bullet

BRITISH Nuclear Fuels PLC has announced that it is planning a £100 million study to find ways of reducing radioactive discharges into the Irish Sea to a level as near zero as possible. The decision was announced last week by Mr Con Allday, the company's chairman, who stressed that the new policy was in direct response to public concern about radioactive discharges from its reprocessing plant at Sellafield (formerly Windscale).

Declaring that the company must respond to "fears which are genuinely held, however ill-founded", Mr Allday said BNF would "not attempt to fight a rearguard action on behalf of rationality". The company would recognize the strength of public opinion and would take steps to reduce discharges to "very much lower levels than hitherto planned".

The company has been through a disastrous few months for public relations. Last November, a television documentary drew attention to a locally increased incidence of leukaemia in children near the reprocessing plant at Sellafield and raised the possibility of a link with the plant, which emits Europe's largest routine discharge of radioactivity into the environment. The documentary spurred an urgent inquiry, now in progress, by a team headed by Sir Douglas Black, a former president of the Royal College of Physicians, and formerly Chief Medical Adviser to the Department of Health.

Days later an unplanned discharge of radioactively contaminated chemical solvent led to public warnings from the government to avoid the use of beaches in the area, a warning recently withdrawn. The company's conduct was later criticized in swingeing terms by the Department of the Environment. More recently, transport unions have threatened to block the delivery of spent fuel to the plant unless the levels of discharge are reduced.

Discharges of both beta-gamma emitters and alpha-emitting actinides from the plant are down to a tenth of the peak levels of the early 1970s. Sir Denys Wilkinson, until recently chairman of the government's Radioactive Waste Management Advisory Committee, said last week at a lecture organized by the Science Policy Research Unit of the University of Sussex that radiation dose to critical groups resulting from the Sellafield operations was "higher than one would like it to be". The original fluorescein experiments on which the discharge policy was based were planned and executed in 4 months, Sir Denys recalled. There had been no full evaluation of the consequences of the discharges, and unforeseen developments such as the lowering of plutonium safety limits and the "uncooperative attitude" of the Irish Sea in returning radioisotopes to land instead of dispersing them into the ocean had reduced margins of confidence. In addition, discharges were unnecessarily high in terms of what is easily achievable. Discharges from the French reprocessing plant at Marcoule, for example, were substantially than that at Sellafield. Sir Denys was nevertheless convinced that nobody had suffered any significant extra hazard as a result of British waste disposal practices, and he expressed doubts as to whether Sellafield could achieve the low emissions reached at Marcoule.

The £100 million study now proposed by BNF is above and beyond current action to reduce discharges costing an estimated £150 million. An ion-exchange plant now nearing completion will cut down discharges of radiocaesium, and a separate salt evaporation plant will substantially cut actinide discharges. Further reductions may be brought about by improvements to the reprocessing plant itself.

The recent events at Sellafield have postponed any possibility of a public offering of shares in BNF, which are at present all held by the government. There have also been extensive changes made at senior management level.

The company is still awaiting the decision of the Director of Public Prosecutions on whether criminal proceedings should be started against BNF over November's debacle. Possible infringements of operating licences were afterwards reported by the Nuclear Installations Inspectorate.

Tim Beardsley

IOS closes lab

THE UK Natural Environment Research Council (NERC) has decided that there is a *prima facie* case for closing the Taunton site of the Institute of Oceanographic Sciences (IOS), which carries out research in sedimentation and applied wave mechanics. The council's governing body was due this week to consider the implications of transferring the work to other NERC sites.

Staff at Taunton are unimpressed with the way NERC has handled the matter. The director of IOS, Dr Anthony Laughton, was said to have promised speedy decisions on the future of the site almost a year ago. Dr Laughton declined last week to make any comment about the proposed closure.

Staff at Taunton do not see where they can go. NERC's budgetary problems mean it cannot put up any new buildings, and there is not thought to be room for existing Taunton staff at the IOS site at Bidston on Merseyside. Most would prefer instead to move to the site of the Institute for Marine Environmental Research at Plymouth.

The accommodation problems will probably be solved by the resignation of many of the staff. Scientific manpower at Taunton is already much less than it was in the past; the decreased emphasis on wave power as a likely future renewable energy source may be part of the explanation. Finance from the Department of Energy has been repeatedly delayed, causing difficulties for those planning research. But, in theory at least, research should not be cut back as a consequence of the move now planned.

Tim Beardsley

Correction

THE limit for nitrogen oxides in ambient air proposed in the EEC directive (*Nature* **308**, 309; 1984) should have read 200 gm^{-3} not 200 $mg\,m^{-3}$. □

Mercury pollution

Dry battery alarm in Japan

Tokyo

A PAIR of headphones and a portable cassette player have become pretty much standard street wear for young Tokyoites, but they are creating a new problem for the Japanese Government — how to dispose safely of the batteries that power them. Alkaline-manganese batteries have been favoured because they provide a stable voltage and last a long time: the trouble is that they contain a lot of mercury which, it is feared, is finding its way into the environment from municipal rubbish tips.

The risk of mercury poisoning raises strong emotions in Japan. In 1956 and 1964, major outbreaks of Minamata disease, caused by eating shellfish contaminated by methyl mercury in factory effluent, left thousands of victims, some paralysed from birth.

Public protest over risks from batteries led this week to an announcement of subsidies and tax incentives for industry to set up a special non-profit research and development association to find ways to make mercury-free batteries. This move has, however, neither been welcomed by industry nor been seen as much more than a sop to public opinion by consumer groups. Industrial companies have been trying for years to take the mercury out of batteries — simply because it is so expensive — but without finding a suitable alternative.

Japan is now manufacturing a staggering 2,850 million dry batteries each year. About two thirds of these are for domestic use and the 70 tons of mercury they contain — mainly in the alkaline-manganese batteries and "button" type mercury batteries used in cameras — end up in domestic rubbish, most of which is used in landfill in 2,500 municipally owned sites. According to calculations at the National Institute of Environmental Science, the research arm of the environment agency, these sites will have an average battery-derived mercury content of about three parts per million — a thousand times higher than the maximum mercury content permitted in water supplies — on top of a small amount of mercury from discarded fluorescent lighting tubes and other sources. As the batteries corrode, the mercury will enter the environment and an unknown fraction of it will be converted into organic mercury by bacteria and leached away. Most sites are small and are converted to other uses within five years; being municipally owned, they are often converted to public use, including the building of schools and playgrounds. The risks are much less from the small fraction of rubbish that goes to incinerators rather than to landfill. Incinerator temperatures are far above the boiling point of mercury and the concentration of vapour reduced into the air is low — much lower than the World Health Organization guideline levels although still thousands of times greater than natural background levels.

Consumer groups are now demanding that batteries be collected and disposed of separately from other rubbish. Already about 400 of the nation's 3,300 municipalities have passed local regulations requiring citizens to deposit their used batteries at special disposal centres rather than in household rubbish, and that number is expected to grow to 800 by the end of the year. All the local authorities can do with the batteries at the moment, however, is to store them until some safe disposal method has been found.

Dr Tsukehiro Gotoo, chief researcher at the National Institute of Environmental Science, hopes to see the construction of five battery reprocessing plants across the country together with the enforcement of a refundable deposit system to encourage the return of used batteries. A private battery reprocessing plant is already in operation at Hokkaido but its charges are high — 80,000 yen (£260) per ton of batteries plus transport charges. Studies at the institute show, however, that setting up regional recycling plants with a capacity of 2,000 million batteries a year would at most add only Y10 (£0.3) to the cost of a battery. Such a move seems more likely to provide a practical solution to the problem than the call of the Ministry of International Trade and Industry (MITI) for research on non-mercury batteries. **Alun Anderson**

Radioactive waste

Mongolian alarm at China's plan

CHINESE plans to act as a "nuclear dustbin" for Western countries have provoked a sharp reaction in the neighbouring Mongolian People's Republic. Alarm was crystallized last month in a formal interview, organized by the Soviet news agency TASS and its Mongolian equivalent Montsame, with a Mongolian physicist, Dr D. Dorjbat, who appears to have been selected as a mouthpiece of grass-roots scientific concern.

According to Dorjbat, the Chinese do not possess a highly developed nuclear technology, and so will not be able to ensure a reliable method of storing the wastes. Since they plan to bury them beneath the Gobi Desert, there is a serious risk of contamination of Mongolian as well as Chinese territory, through seepage of radioactive material in the groundwaters.

Dorjbat went on to discuss the political implications of the plan . The Chinese, he alleged, not only hope to obtain additional hard currency from the plan (their preliminary tenders offer to take up to 4,000 tonnes of waste by the end of the century thus earning $6,000 million); they also want to process the waste into plutonium for nuclear weapons. Numerous "foreign experts", he said, have drawn the same conclusion.

Dojbat made no attempt to explain how, at the same time, the technological level of the Chinese could be too low to deal with wastes but high enough to extract plutonium from those wastes. He seemed more at ease with his political statements, alleging that China is at present helping Pakistan to develop nuclear weapons (a claim which the Pakistanis vehemently deny). He also attacked the "disrespect" for the destiny and lives of the Chinese people shown by their leaders who, he claims, regard a nuclear war as winnable.

Beijing, so far, has made no direct reference to the allegations, but appears to be treating them as significant signals in the current war of words with Moscow. Within a few days, *The People's Daily* carried what was clearly a direct refutation of the attacks on the waste disposal plan, affirming that Chinese technology was well-equipped to deal with all forms of nuclear waste, solid, liquid or gas. The more sinister charges that the Western wastes would be used as the raw materials for bombs was, however, pointedly ignored. **Vera Rich**

Moscow visit off

Washington

AMID continuing uncertainty about the health of Soviet physicist Andrei Sakharov and his wife Yelena Bonner, the US National Academy of Sciences confirmed last week that its president had postponed plans to visit the Soviet Union to re-establish formal links with the Soviet Academy of Sciences.

In a short telegram to A.P. Aleksandrov, president of the Soviet Academy, Dr Frank Press, president of the US National Academy, said talks on a new agreement were "impossible" given the deepening concern of American scientists over the circumstances of Dr Sakharov. The telegram added: "I have delayed until the last moment a final decision in the hope that the situation would be positively resolved in a manner that all of us would recognize. Since this hoped-for development has not occurred, I believe it is to our mutual advantage to postpone the visit of our delegation until a climate more favourable for positive discussions exists."

Although academy officials stressed that the decision to cancel the planned visit was taken by the academy alone, they acknowledged that Dr Press had consulted the government. The officials also stressed that the visit had been postponed rather than cancelled, and hinted that it could go ahead if the Soviet Academy showed signs of interceding on behalf of Sakharov and Bonner. Peter David

Nuclear winter

ICSU project hunts for data

AN urgent call for information on the consequences of large ground-based fires for the atmosphere of the Earth has gone out from the workshop held in Leningrad between 14 and 16 May on the climatic and biological consequences of nuclear war. The meeting is part of the programme by which the Scientific Committee on Problems of the Environment (SCOPE) of the International Committee of Scientific Unions (ICSU) proposes to produce a comprehensive report on the subject by September 1985.

Participants at the Leningrad meeting explain that one of the obdurate uncertainties of the prediction of sharply reduced and even sub-zero temperatures on the surface of the Earth after a nuclear war, called the nuclear winter, concerns the likely behaviour and effects of the large amounts of smoke from fires that would be carried into the atmosphere. The aggregation of smoke particles is, in particular, not taken into account in the models of the effect so far published.

At the Leningrad meeting, Soviet scientists suggested that data bearing on this question might be gathered from studies of large Siberian forest fires, to which end a meeting on the subject is to be convened in the Soviet Union, followed by another in West Germany.

The possibility was also canvassed of collecting data from the forest fire that has been raging during the past two years in Indonesia. One difficulty appears to be that the resolution of satellite data, both spatially and in time, may not be sufficient for the model builders. The SCOPE office responsible for the project thinks it would be valuable to have the services of an aircraft to make observations of major fires occurring in the next twelve months, but is not hopeful that it can find the resources.

On the design of climatic models, the Leningrad meeting emphasized the importance of full three-dimensional models of the general circulation taking account of the "scavenging" of particles from the atmosphere. One of the particular difficulties to be surmounted, according to a statement after the Leningrad meeting, is that of accommodating mesoscale (intermediate scale) atmospheric turbulence in the general circulation models.

The reactions of participants at the Leningrad meeting to the general question whether nuclear winter is a probable consequence of a major nuclear exchange appear to be mixed. Some stress the need for more data and better model studies. One Western participant said, however, that he had been impressed that "the nuclear winter is becoming more real all the time". Some claimed to have detected a difference between what they called the Moscow school (represented at Leningrad by V.V. Aleksandrov), and the Leningrad school (K. Ya. Kondrat'ev), with the former being more certain than the latter that a nuclear winter would follow a nuclear war.

The next workshop within the SCOPE project will be held in Paris in October. Drafting of the final report is due to begin at a meeting in Colorado next January, but the final version of the report will be completed only at a meeting of the project team arranged for next June at the University of Essex in the United Kingdom

Part of the problem that lies ahead is financial. Most of the support for the SCOPE project, launched at the Cambridge meeting of ICSU two years ago, consists of a grant of $75,000 from the Rockefeller Brothers' Trust, but other possible sources are being approached. □

AIDS development

NIH to license HTLV

Washington

THE companies selected by the US federal government to manufacture a blood test for acquired immune deficiency syndrome (AIDS) are expected to be announced this week. A scientific panel has already met to review the 20 or so proposals received in response to an announcement in the *Federal Register* on 3 May, and recommendations have been forwarded to Assistant Secretary for Health Edward Brandt.

The selected companies will be provided with Dr Robert Gallo's method for mass-producing human T-cell leukaemia virus III (HTLV-III) in return for a royalty payment to the government that is expected to be 5 per cent. The *Federal Register* announcement specified that applicants should have experience with retroviruses, virus production and the manufacture and distribution of radioimmunoassay kits, and have access to a P-3 containment facility. Although skill in recombinant DNA techniques was also mentioned, members of the scientific panel that reviewed the applications said they gave far greater emphasis to ability to manufacture the product quickly and in large quantity. Millions of test kits will be required each year to screen blood products. It is expected that the initial production will rely exclusively upon existing technology — growing the viruses in bulk to produce enzyme-linked immunoabsorbent antibody (ELISA) tests to detect the virus. This widely used technique provides a sensitive and simple means of assaying serum antigens. Eventually, recombinant DNA techniques may be used to produce virus antigen for the tests.

Abbot, DuPont and Roche are said to have submitted strong proposals. More than one company is expected to be chosen.

Genentech has submitted a proposal through its joint venture with Travenol, Genentech-Travenol Diagnostics. Genentech is also known to be seeking a basic research collaboration with Gallo and perhaps also with the French group at the Pasteur Institute. Gallo is said to be in something of a dilemma over the possibility of collaborating directly with a private company; on the one hand he needs the expertise in gene expression that the private companies have, but he is also concerned about giving preference to one company outside the context of the licensing agreement.

Stephen Budiansky

Nature index of biotechnology stocks

12-Month high	12-Month low	Company	Close previous month	Close 31 May	Change
14	8	**Biogen (Switzerland)**	11¾	9	−2¾
2	1⅜	**Bio-Logicals (Canada)**	1¾	1½	−½
14⅛	8	**Bio-Response (USA)**	13⅛	8⅞	−4¼
14	10	**Cetus (USA)**	12¼	10¼	−2
10⅜	5	**Collaborative Research (USA)**	7	5⅜	−1⅝
19⅞	11½	**Damon (USA)**	15⅞	11⅞	−4
26¼	12¼	**Enzo-Biochem (USA)**	16	14	−2
10⅛	5¼	**Flow General (USA)**	7	6⅛	−⅞
42¼	28¾	**Genentech (USA)**	35½	29½	−6
10¾	5⅝	**Genetic Systems (USA)**	6⅝	5¾	−⅞
17¼	8¼	**Genex (USA)**	12½	9⅛	−3⅜
23	12¼	**Hybritech (USA)**	17½	12⅛	−4¾
16¼	8¾	**Molecular Genetics (USA)**	11½	9½	−2
15½	10	**Monoclonal Antibodies (USA)**	14	10¼	−3¾
60⅞	43	**Novo Industri A/S (Denmark)**	51⅜	43½	−8⅛
22¾	14½	**Pharmacia (Sweden)**	17⅛	14½	−2⅝

Closing prices are for the last Friday of the month. For over-the-counter stocks, bid price is quoted; for stocks on the American and New York exchanges, the transaction price. *Nature*'s weighted index of biotechnology stocks stood at **135** on 31 May, compared with **164** a month earlier. Data from E.F. Hutton, Inc.

Science in India

The supplement on Indian science Nature *12 April (pp. 581-600) has prompted many reactions. Some are published here, with more next week.*

SIR — The key to considering science and technology in India lies in recognizing that India is trying to go through three revolutions simultaneously — the agricultural (still incomplete), industrial and computer revolutions — with a government that is democratic, a population less than half of which is literate and resources that are hardly adequate for a task never before undertaken. This is the reason for the wide spectrum from poverty to excellence that one finds in India in all aspects of life. I do not think that *Nature*'s supplement recognized this, explicitly at any rate.

A few careless statements crept in — for instance, "India... often describes itself as the third nation in the world of science and technology". The truth is that India does state frequently that it has the third largest scientific manpower, but mainly to illustrate that despite that fact, productivity is low in science, industry and everywhere.

But my main objection is to the way you depicted the central universities as "breeding grounds for violence", apparently on the basis of the report on the working of the central universities. The report has nothing to do with the actual work of the central universities and much less with science in them; rather it is concerned with factors preventing the central universities from working as they ought to. The report is in no way a guide to what the central universities are, any more than a pathologist's report reveals a man's personality.

In fact, the original concern of the report was precisely that of "finding some way to restore authority to where it belongs". For several decades, long before the Indian Institutes of Technology started to blaze their trails, the central universities, notably those of Delhi, Banaras and Aligarh, contributed much to science and humanities. It is totally wrong to say "nepotism, disorder (even violence) and maladministration are rife even in the seven (central) universities...", wherever this information came from.

Your sampling of the scientific institutions and scientists also appears to be inadequate. Unless one is careful, the visible and the vociferous have a high probability of looming large. But surprisingly, after all that is said, you have not given much, except, perhaps, a few more counts in the *Science Citation Index*. Yes, ambivalence seems to be a part of our relations.

But despite these complaints, and although I do not always agree with your conclusions, your survey is the most incisive analysis of the complex system of science in India. I agree with you that "India has by far the best chance of succeeding" in the development of science and technology, not because of "the ingenuity and articulateness of its people", but because, as Aldous Huxley pointed out (*Science and Literature*), one of the greatest achievements of science is to have developed a method independent of those who work with it.

B. S. RAMAKRISHNA
University of Hyderabad, Hyderabad 500 134, India

SIR — You are, of course, right in saying that all is not well in India's Science. But great strides have been made. As our Prime Minister declared during a White House reception, India is engaged in the stupendous task of moving hundreds of millions of people into the twentieth century. Against this backdrop one can understand why our laboratories and classrooms are no longer sheltered. The attendant erosion of academic freedom, loss of objectivity and so on are inevitable, but, one expects, temporary phenomena.

Notwithstanding the problems, there have been some achievements (as noted by you also). The green revolution is a reality, and our chemical industry is able to lean on our own research and development. Where high technology is concerned, we can certainly claim we know how to launch a satellite and how to build a nuclear power station. On the more exotic basic research side, we cannot boast of a string of spectacular discoveries, but then our total investment relative to that in the West is paltry. If achievement is measured relative to obstacles overcome, our record measures up favourably to those of others.

All the same, we are neither satisfied nor complacent. Recently our two academies held a joint brainstorming session to examine how to improve matters. It was discovered (as expected) that streamlining Indian science was about as easy as holding a plasma at a million degrees in a container at laboratory temperature, and for essentially the same sort of reason. But some suggestions did emerge and they have been transmitted to the Science Advisory Committee to the Cabinet.

We are confident we will make it and are working hard towards that objective. As you remark, "the doubt is not whether but when". Meanwhile, our expatriate friends could note that Indian science cannot be bettered by simply asking "Is this all we have done . . .?" (Incidentally, what have those giving this advice done for us?) Nor will it be accomplished by listening to seminars by scientists coming for a family reunion or even under the auspices of an international committee.

G. VENKATARAMAN
Reactor Research Centre, Kalpakkam 603 102, India

SIR — Readers of your survey are likely to conclude that most of what is good and worthy of its name in Indian science is so because (1) a director or a chairman or perhaps a minister knows and means his/her business; (2) they or their organizations have or at least had good overseas connections and (3) they have somehow managed to avoid the anything-but-beautiful influence of the run-of-the-mill scientific workers and of the people at large.

The major contradiction in Indian science is that of its patch excellence *vis-à-vis* its unrootedness in Indian soil. Co-operation from scientifically advanced countries is necessary and should be welcomed. India neeeds to ensure a certain degree of immunity against the crisis, of which there are visible signs, by directing science in international directions. But the country also needs help in promoting the search for identity of the "two Indias".

With the general elections expected this year, the political undertone of the report carries significance. However "surprising" and "dangerous" it may be for Indian science to be strongly influenced by the people in power, in fact its infective power seems to defy territorial confinement.

Lastly, for the sake of correctness of information, the Bose Institute of Calcutta is not and never was engaged in training graduates, nor is there any emphasis on nuclear physics in its research programmes. Neither of the two Boses received Nobel prizes, on which point there seems to be some misunderstanding (p.589-90).

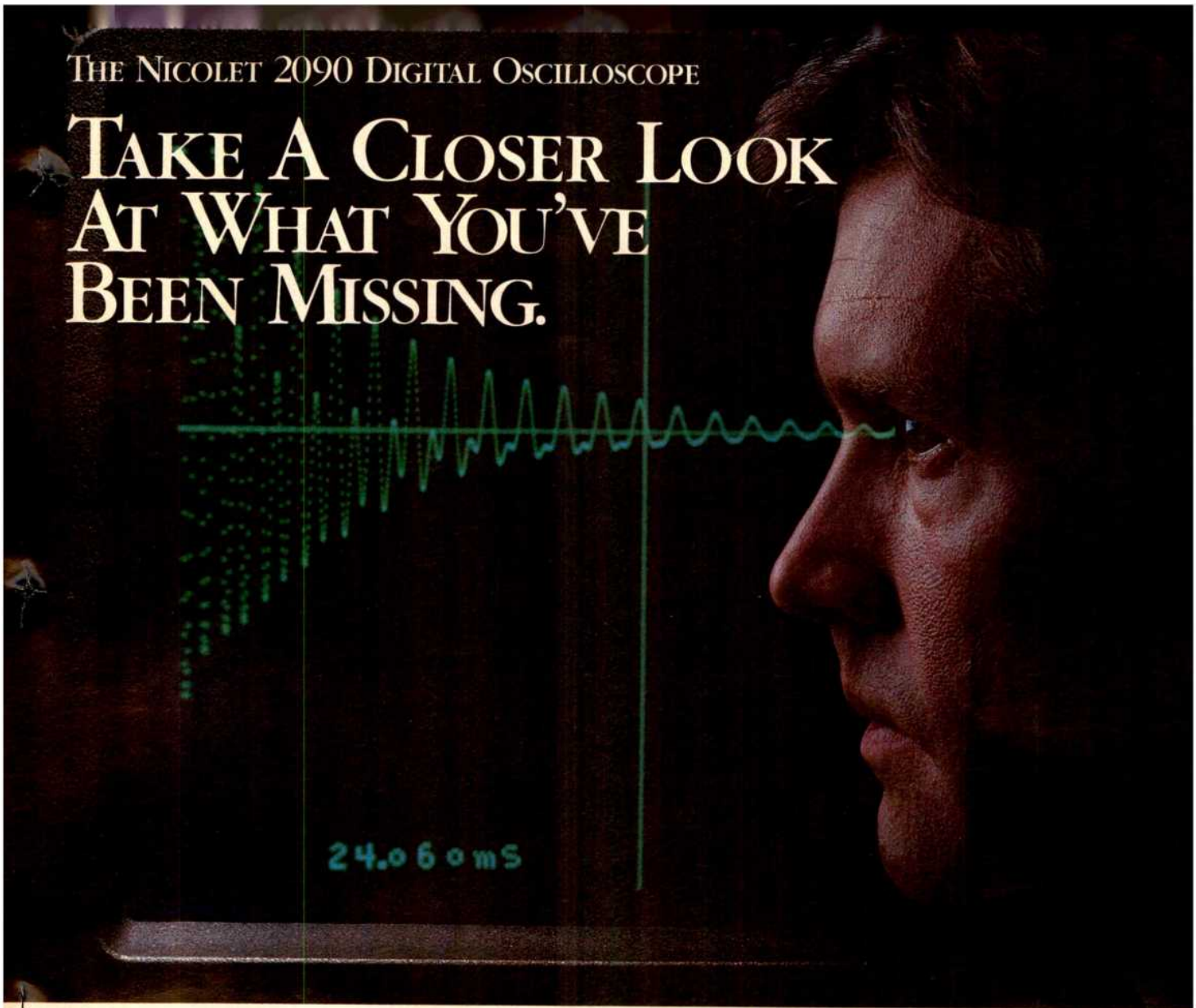
R. MAJUMDAR
Department of Ceramics, University of Leeds, Leeds LS2 9JT, UK

SIR — Your special issue on India provides a comprehensive account of accomplishments, failures and problems of science in India. The introductory note to the survey, however, contains the erroneous figure of 2.5 per cent as the annual rate of India's current population growth. The registration of births and deaths in India is far from complete, but the annual population growth rate of 2.2 per cent during 1971–81, derived from the census counts of 1971 and 1981, is considered by demographers as reasonably accurate. In a recent statement, Prime Minister Indira Gandhi gave the current annual growth rate as 1.9 per cent. The figure of 2.5 per cent instead of 1.9 per cent in the article does not, of course, affect its content or quality, but for India it means an increase of 19 million people every year instead of 14 million. Rapid population growth is a serious problem that Indian scientists and policymakers must tackle.

MONI NAG
The Population Council, One Dag Hammarskjold Plaza, New York, New York 10017, USA

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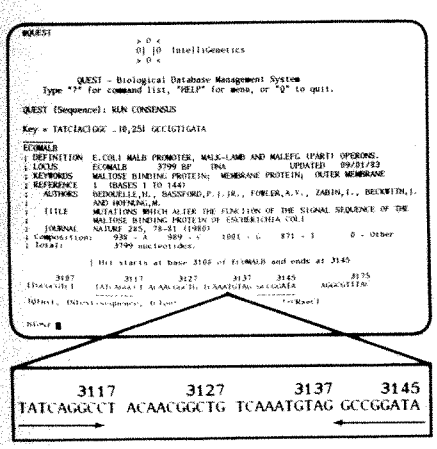
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Sequence Pattern Recognition Program Instrumental in the Discovery of a Unique Sequence Pattern in the Procaryotic Genome

SUMMARY:

QUEST, a sequence pattern recognition program developed by IntelliGenetics, allowed scientists at the Institute Pasteur to rapidly search all available nucleic acid databases for characteristic nucleic acid sequences initially observed in *E. Coli.* The scientists were able to discover the location of 67 members of a family of dispersed repetitive palindromic DNA sequences unique to the procaryotic genome.



3117 3127 3137 3145
TATCAGGCCT ACAACGGCTG TCAAATGTAG GCCGGATA

These palindromic sequences could form stem and loop structures in RNA or be important recognition sites in DNA. The stem is composed of eight remarkably conserved base pairs separated by an intervening region that varies widely in length and sequence.

METHODS:

QUEST's sophisticated query formatting capabilities required only 38 sequence keys to represent over 2.3×10^{17} possible ambiguous sequences. These simplified keys were essential to the rapid search of the entire NIH GenBANK™ and EMBL DNA sequence databases. For example, the pattern for the palindromic repeat "TATC[AC]GGC N{0, 25} GCC[GT]GATA" represents over 6×10^{15} possible different sequences. Additional levels of complexity and ambiguity may be easily represented in the QUEST searching patterns.

QUEST is based on a rapid pattern searching algorithm. Any pattern of either nucleic acid or amino acid sequence can be simply expressed regardless of the naturally occurring degree of ambiguity.

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RESULTS:

Using the 38 QUEST keys, it was discovered that a characteristic 8 base pair inverted repeat (IR) sequence was found predominantly in procaryotic genomes. These palindromic IR's are present at least several hundred times in the genomes of *E. Coli* and other bacteria. These IR's are found primarily between genes and occur either singly or in clusters.*

CONCLUSION:

These inverted repeat regions, characteristic of procaryotes, support the cassette model of genomic evolution, and furthermore suggest a specific role for these sequences in genetic regulation in procaryotes.

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*Accepted for publication in the European Molecular Biology Organization Journal, Vol 6, Number 3 (1984).

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Genetics and hereditable IQ

A new study of IQ among adopted siblings points to hereditable intelligence, but inconclusively. Geneticists (and others) should not however be fearful of a determination of the question.

Is human intelligence genetically determined, environmentally conditioned or the product of both influences? Since the work of the late Cyril Burt was discredited some years ago, the ferocity seems to have gone out of the argument on this question that had been raging for close on half a century. The article that appears on p.620 of this issue, yet another piece of evidence that is by itself inconclusive, will in no sense reawaken that argument. It may however be an appropriate occasion for asking what would happen if ever the question were finally decided.

What T.W. Teasdale and David R. Owen have done is to attempt to discover in measurements made with genetic siblings brought up separately, usually as a consequence of adoption, the degree to which intelligence (as represented by measured IQ) is genetically determined. The study has been made possible by the accessibility of the adoption register in Denmark, and by the ready availability of data on educational attainment, IQ and height (as well as other physical attributes) for males who must register for military service at the age of eighteen. The availability of these data is in part a justification of the pleas that social scientists have been making in recent years that legislation to protect the privacy of individuals should not disallow access for the purposes of legitimate research.

The numbers involved in the study now reported are comparatively small. Five groups of sibling pairs (all male, since women do not register for military service) have been identified, of which two groups have been used, for practical purposes, as controls. One of these groups comprises genetically unrelated males adopted by the same parents and brought up together, the other a larger group of genetic brothers brought up by their natural parents. One essential element in the design of the study is the use of measurements of height (at the age of eighteen) as a kind of control variable. There is a wealth of earlier evidence to suggest that height is indeed heritable, so that height and IQ should behave similarly if, indeed, IQ is inherited.

The outcome of the study, so far as it goes, favours the hypothesis that intelligence is indeed genetically inherited. This, at least, is the outcome of a correlation between the IQs of adopted siblings reared apart. The standard calculation that this result may have arisen by chance yields a satisfactorily low value — the odds are one in 200. But as with other studies of this kind, only some of the crucial questions that will arise in discussion of these data have at this stage been taken up. How comparable were the families into which the several groups of children were adopted? May there not be systematic differences between the families adopting related and unrelated siblings (other than the observation by Teasdale and Owen that parents adopting more than one child are likely to be well off)? One odd feature of the data now reported, however, is that the expected correlation in height among separately reared siblings does not unambiguously show up. The explanation may be the small size of the groups of adopted children, but until other explanations are excluded, the precise significance of this study cannot be certain.

None of this implies that the study has no immediate bearing on the question of whether intelligence is heritable. On the contrary, and at the very least, the study is an imaginative pointer to a source of data on heritability that has previously been almost entirely overlooked. One especially valuable feature of the Danish data is that the records of adoption and the measurements of IQ have been carried out for quite different purposes than that of the authors. Even if Burt's studies of genetic inheritance in identical twins had not been discredited by the suspicion that they may have been crudely fabricated, the fact that he was personally responsible for the measurement of IQ would by now have put his claims in doubt. One of the immediate consequences of what Teasdale and Owen have done will be to stimulate a search for other objectively gathered blocks of data that may throw light on the heritability of intelligence.

But why bother? One common assertion is that when a positive result (in favour of heritability) would be socially divisive, no good purpose can be served by answering the question. Such a response cannot however be regarded as a serious impediment to serious enquiry. If indeed IQ were shown unambiguously to be genetically determined, the result would be interesting in its own right and stimulating of other investigations. And even if it were the case that IQ is only a crude representation of what should properly be understood by intelligence, it would be even more remarkable that such an artefact, constructed from the ease with which individuals carry out artificial tasks, should be genetically determined than that unmeasured and perhaps unmeasurable intelligence should thus be determined.

Precisely what the practical consequences would be of a conclusive determination of the hereditability of IQ is another question. In the ancient row between the nature and nurture schools, it has commonly been assumed that the truth must be uncomfortable for one side or the other. That view should carry very little weight. For nobody pretends that if IQ is inherited, the effects can be as simple as in, for example, the inheritance of well-known physical disorders where a single gene may be responsible.

Everybody agrees that IQ, if inheritable at all, is determined by several genes, perhaps even indirectly. The result is that the IQ of the offspring of parents of known intelligence will be determined by some distribution spanning a range of measured values. Offspring of high-IQ parents will frequently have low IQs. Exactly the same kind of overlap is found in the distribution of IQ in racially different groups of children attending the same schools.

The consequence of these expectations of how measured IQ is likely to be distributed is that even when the question of heritability has been settled, few useful guides to social policy will have been provided. It will remain the case, for example, that societies that insist on educating the offspring of successful parents separately from others, on the twin assumptions that success denotes intelligence and that all offspring of intelligent parents are themselves intelligent, will imperfectly satisfy the needs of many of the children. It would be similarly profligate of human potential to suppose that, if IQ is heritable, schooling as such might be abolished on the grounds that native wit will make itself apparent.

In the whole field of social policy, exactly similar considerations apply. A determination of the question whether IQ is heritable would be largely irrelevant to policy, which is not to say that it would never be used as a means of justifying irrational prejudices. This is why studies such as those of Teasdale and Owen are warmly to be welcomed, why there is the strongest case for hoping that the issue will soon be clarified — and then for learning something of the mechanism by which such a fuzzy attribute of the central nervous system as intelligence may be, even in part, heritable.

John Maddox

Cancer chemotherapy

DNA amplification and multidrug resistance

from Piet Borst

SINCE 1978, when Robert Schimke and his co-workers showed that it could be associated with the development of drug resistance in eukaryotic cells[1], gene amplification has been shown to underlie resistance to several drugs and toxic compounds in a variety of eukaryotic cells, including such unicellular organisms as fungi and protozoa (see ref. 2 for an excellent recent review). There have also been hints that it underlies another form of drug resistance in mammalian cells — the intriguing multidrug resistance (or pleiotropic drug resistance) in which the induction of resistance to one drug leads to cross-resistance to a motley assembly of other drugs with widely different chemical structures and cellular targets[2-6]. Direct evidence that multidrug resistance is due to gene amplification has been missing. That lacuna is filled by the paper of Roninson *et al.* on page 626 of this issue[7].

Schimke's studies were on the development of resistance to methotrexate (MTX), an inhibitor of the enzyme dihydrofolate reductase (DHFR). Resistance to it can arise in at least three ways[3,4]: by a decrease in the carrier-mediated uptake of MTX; by the production of an altered DHFR; and by the increased production of normal DHFR. Schimke and co-workers showed that the third route involves an amplification of the structural gene for DHFR[1].

How does the genome know that DHFR has been blocked by MTX? The answer seems to be simple: MTX inhibits cellular DNA synthesis; when the block is released by removal of the drug, chromosome segments that have partially replicated will not only continue the old replication round but also re-replicate, producing an extra copy of that region. Once gene amplification is underway, selection with MTX ensures that cells with the largest number of copies of the *DHFR* gene will have the best chance of surviving. By stepwise increase of the drug concentration, one can eventually obtain cells with many copies of the gene. (For more on the relationship between amplification and replication the reader is referred to David Denhardt's article in these columns last week[8].)

The extra gene copies produced by amplification may either be re-inserted back into chromosomes to yield homogeneously staining regions (HSRs) or remain as extra 'minute' chromosomes, often found in pairs as double minutes. HSRs are stable when cells are grown without drug, but maintenance of double minutes usually requires continuing drug selection because they lack a centromere. Random distribution of double minutes among daughter cells and loss to the cytoplasm rapidly leads to the emergence of cells that have lost most or all of their double minutes; this instability is a hallmark of drug resistance due to this type of gene amplification (see refs 2, 9–11).

The presence of double minutes in multidrug-resistant cells, as well as the unstable nature of multidrug resistance, has strongly indicated that this, too, is due to gene amplification[12,13]. Resistance of this type can be induced with colchicine, a drug that prevents tubulin polymerization, or with one of the carcinostatic anthracyclines, drugs with diverse effects on cells. Cross-resistance develops to a group of drugs whose only features in common are a molecular weight between 350 and 1,250 and an amphiphilic aromatic nature. This indicates that multidrug resistance is due to altered drug transport, but whether the mutation results in decreased drug entry, increased efflux or a combination of these mechanisms is still not certain[2-5].

A corollary of this type of drug resistance is the overproduction of a large membrane glycoprotein with a molecular weight around 170,000. A reasonable hypothesis is that this membrane glycoprotein is encoded in the amplified DNA[13]. This cannot be the whole story, however, because different multidrug-resistant cell lines show different degrees of cross-resistance to individual drugs.

The direct evidence for gene amplification in multidrug resistance provided by Roninson *et al.* in this issue of *Nature* comes from the use of an elegant, in-gel, renaturation technique. The authors show that two multidrug-resistant Chinese hamster cell lines contain amplified DNA segments, absent in revertants. They have cloned one of the amplified segments as recombinant DNA in *Escherichia coli* and show that the degree of drug resistance correlates with the number of copies of this segment in the DNA of resistant cells. The two cell lines have different patterns of cross-resistance, and it is therefore of interest that the two lines have only part of the amplified DNA segments in common. This suggests that at least three proteins can contribute to multidrug resistance: one preferentially induced by anthracylines, one by colchicine and one induced both by anthracyclines and by colchicine. It should not be too long before the gene products of these amplified genes have been identified.

What is the relevance of this sophisticated molecular biology to the clinician treating cancer patients? Only limited information is available to answer this question. Amplification of the gene for DHFR has been shown to occur in tumour cells from patients who have had prolonged treatment with MTX[14-18]. Multidrug resistance is frequently observed in patients, but whether gene amplification contributes to it is not known. Once the amplified genes in multidrug-resistant human cell lines have been cloned it should be easier to test for amplification of these genes in tumour biopsies by standard molecular and cytological hybridization techniques. I expect that these experiments will give positive results in a significant fraction of patients exposed to intensive treatment with carcinostatic drugs. Most carcinostatic drugs block DNA replication; some of these drugs act on DNA in a similar fashion to certain carcinogens which are among the most effective inducers of gene amplification[1, 19-21]; and the schedules of drug administration used by clinicians are not incompatible with induction of gene amplification.

If gene amplification is important for clinical multidrug resistance, a more complete understanding of the phenomenon might be useful. Knowledge of which gene is amplified could allow prediction of cross-resistance to other drugs. Knowledge of the detailed mechanism of drug exclusion could lead to the design of modified drugs not affected by the exclusion mechanism. The membrane proteins overproduced could form useful targets for antibody-coupled toxins. Multidrug resistance is obviously an area where gene amplification will induce research amplification. □

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Mathematics

Soap bubbles and catastrophes

from Ian Stewart

MANY mathematicians think in pictures, and maintain that geometric ideas offer some of the most important insights in the subject. Others, who think in symbols, disagree. Be that as it may, a number of topics in the mainstream of current mathematical research have clear links to geometry. A recent example[1] combines ideas from the theory of minimal surfaces with Thom's theory of catastrophes[2-4] to shed new light on an old problem posed by soap bubbles.

The study of minimal surfaces goes back to the (blind) Belgian experimentalist Joseph Plateau, who in 1873 published a book, *Statique Experimentale et Théorique des Liquides soumis aux seules Formes Moleculaires*, dealing with the shapes assumed by soap films under various constraints. In particular he noticed that if a closed loop of wire is dipped in a solution of soap and glycerine, a surface forms, spanning the wire. Many elegant shapes can be constructed in this way. If gravity is ignored, such a surface will minimize the energy due to surface tension, that is, its area. Hence the name 'minimal surface'. The 'plateau problem'[5] asks for a rigorous formulation of this concept, and a proof that an arbitrary loop can be spanned by a minimal surface. It has been solved, in various settings (for example, refs 6–8); it has also been considerably generalized.

Catastrophe theory, on the other hand, deals with discontinuous changes in the state of a system having an underlying continuous structure. In its simplest and best known version, elementary catastrophe theory, it studies systems that minimize some energy-like potential function. The best known example, the cusp catastrophe, provides a universal description of the local competition between two stable states. Despite criticism of some speculative models, catastrophe theory has proved applicable to a variety of problems[9,10], though not always those originally envisaged by its creators. Now another area of application has emerged to augment the ever-growing list, and to confound further the pessimists.

About a decade ago, Poston observed that soap films appear to undergo catastrophe-like changes when the loop that they span is slowly deformed[11]. Specifically, if a loop is constructed in roughly the shape of the seam on a tennis-ball, the film will span two stable minimal surfaces (by symmetry); but if it is opened out into a flat circle, it spans a unique surface. Poston argued persuasively that the transitions between these competing states should be described by cusp catastrophe geometry, but gave no proofs. This conjecture has now been demonstrated rigorously by Beeson and Tromba[1].

They find it convenient to work with a specific shape of loop, yielding a surface known classically as 'Enneper's surface'. The variational nature of the problem (minimizing area) is highly reminiscent of catastrophe theory (minimizing potential), but there are technical difficulties. In particular, catastrophe theory is usually formulated for a finite-dimensional set of states, whereas minimal surface problems require an infinite-dimensional set. Nevertheless, Tromba has developed techniques[12,13] to render catastrophe theory applicable to the minimal surface problem, although a number of obstacles must still be overcome. The results add to the available knowledge on a difficult and important problem: how many minimal surfaces are there that span a given loop? Indeed, they furnish the first explicit example of a non-unique solution. The results also contribute to the generalization of catastrophe theory to variational problems in infinite dimensions, thereby bringing several new areas of application within its scope. □

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Botany

St Helena Ebony tree saved

from Mark Williamson

ST HELENA is probably best known as the oceanic island to which Napoleon was exiled. To biologists it is also known for its extinct flightless birds and remarkable endemic trees. It is also a classic example of the destruction of the biota of an oceanic island by man[1]; the introduction of goats and other species and the stripping of bark for tanning have all contributed to the decline of the native flora. Therefore, particularly as 1984 is Plant Year of the World Wildlife Fund, it is a pleasure to record that about 12 species endemic to St Helena are now being cultivated[2], including one that had been thought to be extinct for 130 years.

The island is a mere 121 km^2, lying in the South Atlantic at 16° south. It is ringed by cliffs rising to 200 m or more, while the centre of the island rises to just over 800 m. It was formed by volcanic action 7–15 Myr ago, in the Miocene. A mere 36 species of flowering plants are definitely native[3,4] and no fewer than 33 of these are endemic. Another 20 or so species may be native, but even with them the list would be remarkably short. An equivalent area in England would have roughly ten times as many species.

Two of the most remarkable of the endemic species are the Redwood[5] and the Ebony, now placed in a genus of their own, *Trachaetiopsis*[6], in the Sterculiaceae, a chiefly tropical family which includes the Cocoa and Kola trees. The nearest relatives of the Ebony and Redwood are the six species of *Trochetia* found only on the islands of Mauritius and Réunion in the Indian Ocean. The Ebony seems to have been a dominant small tree in the lower arid parts of the island, while the Redwood was found in the more mesic zone higher up[7].

Whereas the Redwood has continued to survive on St Helena, albeit now only in gardens, the general assumption had been that the Ebony became extinct sometime after the 1850s. But in November 1980, George Benjamin, a forest guard, and Quentin Cronk, of the University of Cambridge, found two bushes of Ebony growing on a cliff[8]. In January and February 1983 Cronk was back on the island with Simon Goodenough, of the Royal Botanic Gardens, Kew. Cuttings from the Ebony were established[2,9] and the plants have now flowered at Kew. The Ebony can be propagated both by cuttings and by seeds, and there are now plans to plant several hundred back on the island[2]. Since all the plants are derived from two bushy individuals, the genetic type and range of the survivors may differ appreciably from the generality of the original stock. But it looks as though the species has been saved. □

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Rheumatoid arthritis

Another viral candidate

from K.A. Brown

DEVOTEES of the school that believes an occult virus is responsible for the induction of rheumatoid arthritis will have welcomed a recent article summarizing a three-year collaborative study undertaken in three USA centres on the identification of parvovirus-like particles in synovial tissue from the joints of rheumatoid arthritis patients[1]. For the sceptics in this field, the publication heralds the arrival of yet another viral candidate whose association with rheumatoid arthritis, if any, will be shown to be circumstantial and which warrants investigation simply because it is one of the few viruses which has not already been the subject of an attempt to associate it with the disease.

The research of Simpson *et al.* stems from their isolation of an infectious agent, termed RA-1, from rheumatoid synovial cells, co-cultured with human lung fibroblasts or rabbit fetal synovial cells, and subsequently passaged in suckling mice. Injection of RA-1 into the cerebral area of the brain of neonatal Swiss–Webster mice produced a fatal neurological disease, whilst its inoculation into the peritoneum produced several symptoms, notably permanent crippling, alopecia, conjunctivitis and dwarfism. Such fatalities and disabling effects were not induced by synovial extracts prepared from patients with the non-inflammatory disorder osteoarthrosis.

In their size (24–25 mm), morphology and resistance to chemical and physical treatment, the infectious particles resemble a parvovirus. Parvoviruses are among the smallest of the DNA viruses. There are two main genera: the adeno-associated, whose replication is 'defective' and requires the help of a co-infected adenovirus; and the adeno-independent, whose replication does not require assistance. In domestic animals parvoviruses are believed to be responsible for such conditions as panleukopenia and enteritis in cats and hepatitis in dogs and geese. Human 'serum parvovirus-like virus' was discovered during the screening of symptom-free blood donors for hepatitis B virus antigen[2]. This adeno-independent virus, often referred to as the B19 virus, was shown to induce febrile illnesses in haematologically normal people[3], aplastic crisis in patients with haemolytic anaemia due to infection of a haematopoetic progenitor cell[4] and to be transmitted to haemophiliacs in blood clotting factor concentrates[5].

Since established laboratory cell lines may be contaminated with parvovirus, there is always a risk of parvovirus contamination during the co-culture and passage stages of experiments. However, Simpson *et al.* show that injection of rheumatoid arthritis synovial cells into murine brains, without prior co-cultivation with accessory cells, induces the appearance of the RA-1 virus. Furthermore, the RA-1 antigen does not react with antisera directed against several other parvoviruses which infect mice and other animals.

On caesium chloride equilibrium gradients, the RA-1 viral particles are found at a density of 1.30 – 1.43 g cm^{-1}. In a collaborative study which is shortly to be submitted for publication, Gabi Stierle, a visiting scientist to our department, demonstrates that caesium chloride fractions of 1.41 g cm^{-1}, prepared from synovial cells of five of eleven rheumatoid arthritis patients, but none of six osteoarthritis patients, react with an antibody against RA-1. The immunoreactive rheumatoid fractions contain abundant particles of 10 nm diameter whilst 23 nm particles are seen in similar isolates from long-term rheumatoid synovial cultures. The rheumatoid fractions do not react with the monoclonal antibodies to the B19 virus. This finding, together with the failure of the B19 virus to react with the anti-RA-1 antibody and the high prevalence of the anti-B19 antibody in 61 per cent of the population[6], suggests that it is unlikely that the B19 virus has an important role in rheumatoid arthritis[1].

Parvovirus is the infectious agent of Aleutian disease in mink, a slowly progressive fatal disorder characterized by hypergammaglobulinaemia, anaemia, immune complex-mediated arteritis and glomerulonephritis[7] and thought by some investigators to have features of autoimmune disease. There is no evidence to suggest that parvovirus may directly initiate a synovitis, though the affinity of the virus for host cells that are of high mitotic activity, particularly activated lymphocytes[8] and endothelial cells[9], and its dissemination by monocytes and macrophages[8] are attributes which might be expected of a polyarthritogenic agent. It is premature to indulge in conjecturing possible implications of the work of Simpson and Stierle for rheumatoid arthritis. We must await definitive characterization of the RA-1 virus by techniques such as cell hybridization, serological evidence of parvovirus infection, laboratory studies performed on patients with inflammatory arthritides other than rheumatoid arthritis, and cytopathogenetic studies using adenovirus and other viruses which might support its replication.

Simpson *et al.* are to be encouraged in their persistent and patient search for a replicating virus in rheumatoid arthritis, a task that seems fraught with almost insurmountable biological and technical obstacles. Whatever the outcome, they will have resurrected interest in an area which urgently requires more motivated scientists of enthusiastic application and original approach. Progress is painfully overdue. □

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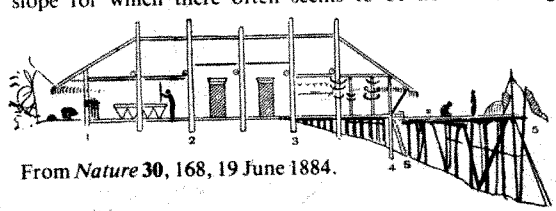
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100 years ago

PILE-DWELLINGS ON HILLTOPS

AMONG the Miris, Singphus, Kamtis, Nogas, Mismis, and Deodhains that I have questioned as to the origin of the custom of building on piles, the answer is invariably that they do not know, that it is their tribal custom, &c. Pressed as to the advantages of it, or why they could not build on the ground like Hindus, they generally end by urging the absolute necessity of keeping things out of reach of the ever-present pig.

As an illustration of how this animal practically affects the question of house-building, I append a section of a typical average Noga house, as built by the tribes south-east of Sibsagar to the Upper Dihing. One end generally rests on the ground, while the other overhangs a slope for which there often seems to be no occasion, as plenty of level land is about. In all houses of this type the end devoted to husking rice rests on the ground, and the door at the end has a slab that can be raised to admit pigs to eat the husks. This compartment (one-third of the house) is divided from the living and sleeping part by a wall and a door with a stile to keep out pigs. There are generally from two to six, or more, sleeping-rooms on the ground, and beyond them again is an open room used for visitors, or to sit and work in during wet weather.



From *Nature* **30**, 168, 19 June 1884.

But there are many other things besides pile-dwellings that prove these now distinct tribes to have descended from a common stock. The "morongs," or houses in which the lads and single men sleep at night, away from their parents' houses, are seen under various names all through these hills. There are also "morongs" for the girls and single young women, and there are special and peculiar laws relating to morongs. Liberty of the sexes before marriage is indeed practically so complete among all these tribes that really *morals begin with marriage*. After marriage they are better, I think, than civilised nations.

Catalysis

Zeolites under control

from Lovat V.C. Rees

ON page 589 of this issue of *Nature*, Haag *et al.* report a very nice example of the way in which zeolites can be used in the controlled catalysis of hydrocarbon cracking, which is best seen in the context of John Dwyer's brief but comprehensive overview of zeolites as acid catalysts in the 2 April issue of *Chemistry and Industry*..

Zeolites are crystals of alumino-silicates permeated by channels and cages of molecular dimensions. The active catalytic centres are hydroxyl groups — one for each Al atom — which are readily generated by deammoniation of ammonium-exchanged zeolites on heating to 400°C. On heating to temperatures greater than 550°C, however, these Bronsted acid sites are converted to Lewis sites by loss of H_2O. John Dwyer clearly indicates the importance of zeolites in elucidating the fundamental mechanisms involved in the catalytic conversion of hydrocarbons. Since zeolites can readily be synthesized over a very wide range of Si/Al ratios, it is possible to create catalysts with active centres whose average separation distance can be varied in a controlled way. Also, by choosing specific zeolite structures from a large list, channel and cage geometry can be varied. Specific channel geometries can also be modified by various techniques.

With this ability to finely tune zeolite catalysts, it is possible to observe the effect of increasing the acid strength on, for example, the product ratios resulting from the isomerization of butenes. The shape-selective behaviour of zeolite catalysts can be readily quantified. Thus reactant and product shape-selectivity and restricted transition-state-selectivity can be explained. The size and shape of the channels and cages exert both geometric and diffusional restrictions. The size of the crystals, coking, steam treatment and chemical modifications affect the products of a reaction in easily explained ways.

It is usually quite difficult to identify and count the number of active sites involved in a catalytic reaction but that is not the case for ZSM-5, the zeolite studied by Haag *et al.* and in which it is possible to have such a high ratio of SiO_2 to Al_2O_3 that the Al atoms are no longer a structure-determining constituent. Haag *et al.* varied the Al content of ZSM-5 from 20 to 10,000 p.p.m. such that the average distance between Al sites represented several to a very large number of atomic diameters and the distribution of the sites will have approached one of randomness. There is, thus, a substantial chemical uniformity to the catalytic sites.

The activity of these samples towards the cracking of *n*-hexane in controlled conditions was linearly related to the Al content and extrapolated to zero activity at zero Al content. This quantitative correlation can be explained only if all Al atoms are in tetrahedral framework locations — which is exactly how they are shown to be arranged by high-resolution magic-angle spinning nuclear magnetic resonance.

With such accurate quantitative data it is possible to determine the number of active sites, the absolute molecular turnover rates and the turnover numbers. Haag *et al.* found an absolute turnover rate for *n*-hexane cracking of 2.8 molecules per minute per atom of Al over the entire Al concentration range. Since the reaction rate is first order, the fraction of occupied Al sites at 100 torr is less than 1 per cent of the total Al sites and the turnover rate per occupied active site (the turnover number) is greater than 300 molecules per minute per active Al site.

The reaction rates for other reactions, for example 1-hexane cracking and 1-hexane double-bond isomerization, have also been shown to be linearly dependent on the Al concentration. The olefin cracking rate is some 800 times the paraffin cracking rate while the olefin isomerization rate is some 1,300 times the olefin cracking rate. The Al concentration needed to achieve technically relevant reaction rates can be less than 1,000 p.p.m. for paraffin conversion and from a fraction of a p.p.m. to 100 p.p.m. for other conversions. Turnover numbers as large as 4×10^7 can be obtained with zeolite catalysts; not dissimilar to the values in enzyme catalysis.

Ultra-stable zeolite Y is currently used as the cracking catalyst in oil refining. It cannot be synthesized with a ratio of SiO_2 to Al_2O_3 greater than 3, but this ratio can be increased by various de-alumination processes. These impart additional thermal stability but lower the crystallinity, and the resulting catalyst is still too active and requires dilution with catalytically inactive binder material. Even then, coking of the catalyst pellets occurs in a reactor in a time scale measured in seconds. The data of Haag *et al.* on ZSM-5 indicate how little Al is necessary for cracking reactions and should spur zeolite chemists on to discover a method to produce zeolite Y with very much less Al than used at present. Very interesting technical problems will have to be overcome to produce such a catalyst.

Finally, Martens *et al.* show that a relatively simple test can be used to obtain a detailed insight into the structure of the channels and cages of zeolites (*Zeolites* **4**, 98; 1984). Using *n*-decane isomerization and hydrocracking reactions over platinum-loaded hydrogen zeolites as a fingerprint, five independent criteria can be found which give consistent and supplementary evidence on the structure and dimensions of the void space in zeolites. Voids that are characterized in terms of unidimensional pores made of 10-membered rings of oxygen atoms are readily distinguished from those that are cages of varied dimensions accessible through pores made of 8- or 12-membered rings. Surely the consistent reproducibility of these catalytic reactions indicates the uniqueness of zeolites as catalysts. □

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Immunology

T-cell development in the thymus

from Ken Shortman

MUCH of the development of T lymphocytes takes place in the thymus, away from the melee in bone marrow where B lymphocytes and other haematopoietic cells differentiate side by side. What is the purpose of this convenient packaging? Is the thymus an inductive environment for switching the differentiation of uncommitted stem cells into one of the T-cell lineages? Or is it an immunological finishing school, generating a diversity in the antigen receptors on the surface of T cells and selecting those most suitable for recognizing foreign antigens presented in the context of 'self' (major histocompatibility) antigens on the surface of cells. Or is it both? A careful study of what goes in and what comes out can set the limits of what might happen in the thymus itself.

Recently devised techniques[1,2] for labelling cells within the adult mouse thymus and for tracing labelled emigrants into the periphery have shown that emigrant cells are much like mature T cells, are already committed to one or other of the specialized T sublineages and are already functional. The essential development of the cells must have occurred within the thymus, or even before their arrival there. The output from the thymus is low, however: only about 3 per cent of the cells produced daily leave the thymus, the rest apparently dying within it. Although this high attrition rate suggests a stringent culling for appropriate specificity, it could equally well reflect a low efficiency in the formation of a functional T cell of any specificity.

The other part of the equation, the seeding of T cells in the thymus, is considered by Ezine, Weissman and Rouse on page 629 of this issue of *Nature*[3]. They have studied the reconstitution of the T-cell

population of the thymus of mice whose haematopoietic stem cells, including those for T cells, have been destroyed by irradiation. For the purposes of reconstitution, Ezine *et al.* supplied the mice with a mixture of a large number of bone-marrow cells from identical mice and a small number from congenic mice that carried a different T-cell surface marker. It was thereby possible in immunohistological studies of frozen thymic sections, prepared several weeks after reconstitution began, to tell which T cells had originated from which stem cells. The results were similar to those that had been previously obtained using a chromosome marker[4]: often thymus lobes contained no cells of congenic type and in other cases discrete foci of congenic-type cells were seen, and occasionally an entire thymus lobe was uniformly of that type. This pattern of distribution indicates that the thymus is recolonized by very few 'stem' cells, and sometimes by only one. As Ezine *et al.* point out, although this model does not tell us the actual number of stem cells colonizing a thymus throughout embryonic development, it does suggest it could be small compared with the size of the T-cell specificity repetoire. That argues in favour of the generation of receptor diversity within the thymus.

The second issue raised by the study is the long-disputed relationship between the thymic cortex and medulla. The medullary region of the thymus contains most of the organ's phenotypically and functionally mature cells (about 10 per cent of the total cell population). The simplest interpretation is that these are the immediate source of cells destined for export, although there could be a similar, but small and transient, pool of such cells in the cortex. The cortex contains the majority of dividing cells and the majority of thymocytes, most of which are both non-functional and phenotypically different from mature T cells. The simplest interpretation would be that these are the immature precursors of the medullary population, but this does not accord with the intrathymic death of most cortical cells. The medullary and cortical populations in fact display a large degree of independence in cell kinetic studies[5,6].

Nevertheless, Ezine *et al.* report cases where both cortex and medulla appear to be repopulated by a single clone, suggesting that a single stem cell has initiated a cell lineage which populates first the cortex and then the medulla. A subpopulation of apparently cortical blast cells, capable of reconstituting both cortical and medullary compartments, was reported by B.-J. Fawlkes and B. Mathieson at the meeting on T-cell differentation at the Basel Institute for Immunology last November. This could be a later intrathymic stage of the type of bone-marrow stem cell suggested by Ezine *et al.* Note, however, that Ezine *et al.* also report instances where only the medulla showed reconstitution with congenic-type cells, suggesting a direct seeding of the medulla without cortical involvement. The studies of Joterau and Le Douarin[7] on colonization of the avian embryonic thymus also suggest independent seeding of the cortex and the medulla.

Why there might be two separate developmental pathways, one cortical to medullary, one medullary alone, is far from clear. It would make more sense if T-cell sublineages of different function were handled in different parts of the thymus. With our present knowledge, it would be more sensible and comprehensible if all T-cell development, from stem-cell immigrants to functional T-cell emigrants, took place in the medulla. It is the involvement of the cortex, with its high level of both cell generation and cell death, and with its non-functional lymphoid cells of peculiar surface composition, that forces us to acknowledge there is something interesting happening in the thymus that we still do not understand. □

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Earth science

Anomalous satellite motion and mantle viscosity

from Kurt Lambeck

FOR many years geodesists have examined evidence for the temporal changes in the Earth's global shape and gravity field. Confirmation that such changes occur on time scales of at least several years has now emerged from the observation of a secular acceleration $\ddot{\Omega}$ of the node Ω of the orbit of the satellite Lageos[1,2], launched by NASA in 1976 and tracked ever since with high-precision laser-ranging equipment. Acceleration of the node implies a secular change in the flattening of the Earth and the most obvious explanation of this — the mass readjustment inside the Earth associated with the rebound of the crust after the melting of the Late Pleistocene ice sheets[1,2] — has recently been analysed in detail by Wu and Peltier[3].

One benefit of the measurement of the anomalous orbital motion is that it will provide an additional estimate of the viscosity of the mantle, a quantity that is central to discussions of mantle convection yet remains uncertain. The information on viscosity contained in the observation of $\ddot{\Omega}$ is the same as that contained in the observation of the Earth's secular acceleration but the latter is contaminated by other physical processes, most notably the secular tidal contributions; as a result no accurate figure for the non-tidal acceleration is available. Furthermore, non-tidal acceleration cannot be uniquely associated with the post-glacial rebound; for example, very long-period contributions could arise from electromagnetic coupling forces acting at the core–mantle interface. The orbital perturbation in the node may therefore reflect a more direct measure of the mantle's effective viscosity.

The power of the analysis of satellite orbits to determine the dynamic flattening, J_2, of the Earth with high precision is well known[4]. Nonetheless, observations of temporaral fluctuations in this quantity have proved elusive. What was missing, until Lageos, was a satellite that could be tracked with great precision and whose orbital motion was almost entirely governed by the Earth's gravity field.

The two studies use nearly the same Lageos data and there are many similarities in the orbital analyses used. Yet their results for the secular accelerations differ significantly: Rubincam[2] obtains $(-10.8\pm2.4)10^{-3}$ arc s yr^{-2} as the value of $\ddot{\Omega}$ while Yoder *et al.*[1] obtain $(-14.7\pm1.3)10^{-3}$ arc s yr^{-2}. Are we in for another long debate on the correct value of a secular variation, matching that of the Earth's non-tidal secular rotation? Why do these differences occur? The problem may be partly one of contamination by long-period tidal contributions because superimposed upon the secular motion of Ω are long-term periodic fluctuations due to the tidal deformations of the Earth, principally the 18.6 year tide. The periods of these tides are well known but the amplitudes are not, primarily because both the ocean response and departures of the solid Earth's response from that of an elastic body remain uncertain[5]. Thus with only 5.5 years of data the Lageos results are still very preliminary. In more leisurely times the authors would have patiently waited until a much longer record had been painstakingly acquired, but one cannot be sure that NASA will still be tracking Lageos a few years from now.

Whatever the exact answer, Wu and Peltier believe it can be explained as the response of the Earth to the new surface load following the removal of the Pleistocene ice from the glaciated continents and the addition of an equal water-mass to the oceans. Locally, sea level and gravity are changing through time; globally the Earth's rotation and the node of satellite

orbits undergo secular changes. Many attempts have been made to deduce mantle viscosities from these direct and indirect observations of the post-glacial rebound but the results have remained conflicting. What can be said is that nearly all observations are accommodated by mantle models of uniform Newtonian viscosity of 10^{21}–10^{22} P. Where controversy arises is in whether there is a significant depth-dependence of viscosity, whether this viscosity is linear and whether there is evidence for lateral variations in viscosity[6]. A principal reason for this state of affairs is that the results are only as good as our inadequate knowledge of the distribution and melting history of the ice loads themselves, partly determined by the rebound observations, particularly by observations of sea-level changes[7-9].

The new J_2 or $\ddot{\Omega}$ observations are largely consistent with the uniform and Newtonian viscosity range of 10^{21}–10^{22} P. Wu and Peltier[3] have attempted to combine this observation with those of the rotational response to deglaciation and conclude that the data imply there to be a thick elastic lithosphere overlying an upper mantle of viscosity 10^{22} P and a lower mantle viscosity of 1–3×10^{22}P. The average thickness of this elastic lithosphere is estimated to be about 120 km with the continental layer being about 200 km thick. This conclusion is reached by assuming a viscosity profile that is determined by local observations of rebound[10] and then determining the lithospheric thickness from the rotation data. The conclusion therefore remains dependent on the assumed ice history. Other recent studies[7-9] have shown that the proposed melting histories remain inadequate to explain sea-level changes in tectonically stable regions far away from the ice loads and point to mantle viscosities that are less than 10^{22} P. As long as the conclusion of a thick average lithosphere is not dependent on the assumed ice load, these observations raise the interesting possibility that there are substantial regional variations in viscosity, for the far-field Holocene sea-level fluctuations are responsive mainly to the mantle flow induced by the added melt-water rather than the flow under the former icesheets. □

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Oncogene activation

Message of *myc* in context

from Miranda Robertson

THERE are two reasons for the exceptional concentration of research on the cellular *myc* proto-oncogene (c-*myc*). The first is historical. The chromosomal translocations characteristic of Burkitt's lymphomas transfer c-*myc* from its normal context into an active immunoglobulin locus[1], where it was quickly identified by a number of research teams whose vigorous investigations on the molecular basis of antibody diversity were immediately deflected to the molecular basis of cancer. The second is the nature of the c-*myc* gene itself. Unlike many other oncogenes, c-*myc* is expressed in a wide variety of both normal[2] and tumour[3] tissues, and can therefore be expected to have some general significance. This expectation is borne out by two recent papers directly implicating c-*myc* in the control of normal and abnormal cell growth[4,5].

The implication of all the recent research, broadly, is that what changes during tumorigenesis is not c-*myc* itself but the regulation of its production. The evidence comes partly from the two recent papers[4,5] on changes in c-*myc* expression during the growth cycle of tumorigenic and non-tumorigenic cells *in vitro*, and partly from the latest investigations on the interaction of c-*myc* with the regulatory mechanisms operating on the immunoglobulin locus in Burkitt's lymphoma cells.

c-*myc* and the cell cycle

The data on the cell-cycle regulation of c-*myc* come from four laboratories: those of Philip Leder and Charles Stiles[4], and those of Gail Sonnenshein and Arthur Pardee[5]. The aim of their experiments was to establish where the product of the c-*myc* gene might act in the successive steps by which cells progress through their growth cycle. There is evidence, cited in both papers, that the progression from quiescence to DNA synthesis (that is, through G_0–G_1)can be divided into roughly three phases, each triggered by different tissue-specific growth factors. The first phase is known as the phase of priming for growth competence; and it is in this phase that c-*myc* is implicated.

Kelly *et al.*[4] have been able to show that a fibroblast mitogen, platelet-derived growth factor (PDGF), and two lymphocyte mitogens, concanavalin A and lipopolysaccharide, stimulate the expression of c-*myc* in those cells that normally respond to them. All three mitogens are believed to act early in the cell cycle to induce competence; and all stimulate transient expression of c-*myc* within 1–2 hours, with a return to baseline well before the onset of DNA synthesis.

The activation of c-*myc* by PDGF is particularly noteworthy, and indeed is particularly noted by Kelly *et al.*, in the light of the very close relationship between the sequence of one chain of PDGF and that of the product of the c-*sis* oncogene[6,7]. This may be the first clear illustration of how the cellular proto-oncogenes encode different components of a single regulatory chain, disruption of any part of which could contribute to tumorigenesis.

The implication of the Campisi *et al.*[5] data is that after a normal cell has been transformed into a tumour cell, regulation of the c-*myc* gene may be disrupted in such a way that its activation is independent of growth factors. They have measured c-*myc* expression during growth and quiescence in three cell lines: the non-tumorigenic mouse fibroblast line A31, and two tumorigenic derivatives, BPA31 and DA31, both transformed by chemical carcinogens. The growth of all three lines can be arrested by starvation (though the tumorigenic cells are slower to respond); but whereas arrested A31 cells cease to express c-*myc*, the transformed cells continue to express the oncogene at the same level after arrest. Apparently, c-*myc* expression, and thus growth competence, is no longer subject to regulation by serum growth factors after transformation.

These observations are, as Campisi *et al.* point out, consistent with the existing evidence that the activation of c-*myc* depends on the disruption of the genetic control of its expression, and not with mutations affecting the function of the product. (This is by contrast, for example, with the c-*ras* family, at least some members of which can be activated by mutations in their coding sequence [8-10].) They are not, as it happens, particularly consistent with the notion, derived from the transfection experiments of Land *et al.*[11], that c-*myc* is one of a class of oncogenes whose activation immortalizes cells (A31 cells are already immortal) and that a second gene (for example one of the *ras* family) is needed to complete transformation. But then, this categorization is in any case operational and indeed Land *et al.* explicitly state that they have no direct evidence that c-*myc* transfection immortalizes. What all this probably means is that tumorigenesis is a function of interactions between different oncogenes (in the Land *et al.* experiments c-*myc* and c-Ki-*ras*) that need not be the same in all tumours. There is, for example, no way of knowing whether the deregulation of c-*myc* in BPA31 and DA31 cells is due to a change in the regulatory region of the gene itself, or to the deregulation of some other component of the pathway in which c-*myc* par-

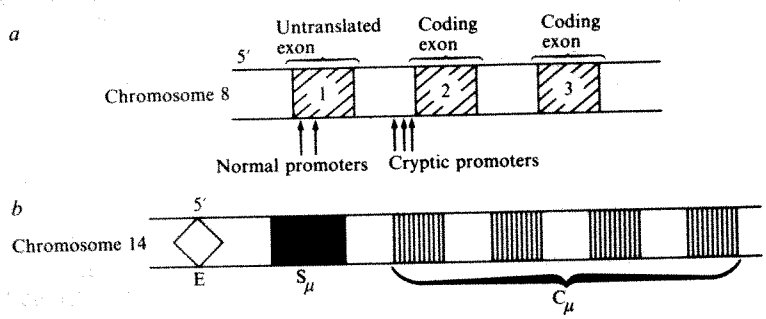


Fig. 1 *a*, The normal c-*myc* gene, which is on chromosome 8 of man, has three exons, the most 5′ of which is untranslated. It is normally transcribed from two promoters at the 5′ end of exon 1: the significance of the two different transcripts for the translational regulation of the normal gene is so far a matter of debate (see ref. 1). When exon 1 is removed from the gene by chromosomal translocation, transcription is initiated at one of a cluster of cryptic promoters within the first intron. *b*, The immunoglobulin heavy-chain locus on chromosone 14 which is involved in a reciprocal exchange with chromosome 8 in most Burkitt's cell translocations. In the normal course of B-cell differentiation, rearrangements of chromosome 14 bring one of several variable-region segments into juxtaposition with the constant-region segment, and thus under the control of the transcriptional enhancer sequence just upstream of it. *E*, enhancer; S_μ, switch sequence (this sequence participates in further chromosomal rearrangements that take place later in B-cell ontogeny but have no direct relevance to the Burkitt's translocations); C_μ, μ constant-region segment, containing four exons (shaded area).

ticipates: it might even reflect changes in c-*sis*.

It is in establishing a precise mechanism for c-*myc* deregulation that Burkitt's lymphoma cells may be important. In those cells, the c-*myc* gene on the normal chromosome 8 is silent, while its allele, which has been translocated to an immunoglobulin gene locus, is constitutively active. Thus deregulation must be associated with the translocated gene itself, or its flanking DNA. The latter could be either from the original context of c-*myc* on chromosome 8 or from its new context in the immunoglobulin locus (usually on chromosome 14).

Mutation and derepression

There have been, very broadly, two schools of thought on the mechanism of c-*myc* activation in Burkitt's cells. The first, which derives from George Klein's original observations on Burkitt's translocations[12], is that the answer lies in the constitutive activity of the immunoglobulin locus in the lymphoma cells. While everyone agrees that the immunoglobulin locus must play some part in c-*myc* activation, it has proved remarkably difficult to implicate any specific immunoglobulin regulatory mechanism: various abortive attempts have been summarized in an earlier article[1]. The alternative is that the c-*myc* gene and its own regulatory sequences are altered in the translocation. There is fresh evidence for both schools of thought.

One possibility that can almost certainly be ruled out is that c-*myc* is activated by mutations in its coding exons. The c-*myc* gene has three exons (Fig. 1*a*), of which only exons 2 and 3 are translated [13,14]. Exon 1, which is highly conserved, is suspected of subserving a regulatory function. In no case is either exon 2 or exon 3 broken in the Burkitt's cell translocations; and with one exception (which will be discussed later) no mutations have been found in the translocated coding exons (ref. 15 and C. Croce, personal communication). Attention has thus recently been focused on the putative regulatory exon, exon 1. The issue is the importance of the loss of *myc* regulatory regions relative to that of acquisition of immunoglobulin regulatory elements, in the activation of the translocated c-*myc* gene.

The evidence in favour of a regulatory function for exon 1 of c-*myc* is strong but circumstantial. Exon 1 is highly conserved across species in the cellular gene, but entirely absent from the v-*myc* oncogene of tumour viruses. This strongly implies that it is vital to the normal function of the gene but unnecessary or even inimical to its transforming function. Leder and his colleagues have put forward the theory that the 5′-untranslated region including exon 1 is the binding site for a transcriptional repressor, possibly induced by, or even identical to, the c-*myc* product itself[16]. This kind of regulatory feedback could clearly account for the transience of c-*myc* activation by growth factors; and Leder and his collaborators have bolstered their case by showing that when protein synthesis is inhibited by cycloheximide, transcription of c-*myc* in response to growth factors is prolonged[4].

The circumstantial case is further strengthened by the chromosomal translocations that are typical of mouse plasmacytomas. Like those in Burkitt's lymphomas, they almost always move c-*myc* to the immunoglobulin heavy-chain locus (see Fig. 1*b*); and in every case, the first exon of c-*myc* is either lost or truncated in the translocation. This suggests that loss or disablement of exon 1, or other sequences 5′ to the coding exons, is associated with the production of tumours. (That the truncated gene can be transcribed at all is due to a copious supply of cryptic promoters in the first intron: see Fig 1*a*.)

Burkitt's lymphomas, however, have failed to comply with this picture of c-*myc* activation: in many of them, the c-*myc* gene is translocated intact (see Fig. 2*a*). This has been interpreted as evidence that the constitutive activity of the immunoglobulin locus is more important than the state of exon 1 of c-*myc* in deregulating the translocated gene. Recent results from Leder's and Rabbitts's laboratories suggest an alternative, arising from a second specialized property of the immunoglobulin locus. Through a mechanism that is still not understood, antibody diversity is increased by the generation of somatic mutations in a discrete region of DNA normally occupied by the variable-region segment of the immunoglobulin gene. In Burkitt's lymphomas, this site is usurped by c-*myc*; and Rabbitts *et al.*[17] have shown that in one case — that of the Raji cell line — the translocated c-*myc* gene has undergone extensive mutation. Leder and his colleagues have now used sequencing and S_1 mapping to demonstrate mutations in the intact exon 1 of two more Burkitt's cell lines[18]; and Rabbitts *et al.*[19] have found mutations in yet another two.

If exon 1 of translocated c-*myc* genes were invariably either mutated or lost, this might be taken to resolve the activation issue in favour of the gene rather than the immunoglobulin locus. There are two complications, however. The first arises from Rabbitts's original data on mutations in Raji cells. These mutations extend into exon 2, the coding portion of the gene, where they result in 16 amino acid substitutions. It is hard to imagine that these would leave the myc product functionally unaffected; yet conserved myc function seemes to be essential to tumorigenesis[15].

This particular paradox is resolved by the activation, in Raji cells, of the untranslocated c-*myc* gene — which is silent in other Burkitt's lines[19,20]. Rabbitts attributes the activity of the untranslocated c-*myc* gene directly to the mutant product of the translocated one, under Leder's repressor theory. If the myc product usually mediates or induces repression of the c-*myc* gene, then a mutant product may lead to a failure of repression. This leads to another paradox, however, since the normal c-*myc* gene once activated ought presumably to induce its own repression. There are various possible ways out of this difficulty, but in the present state of knowledge they are all more or less arbitrary.

The second complication arises from some data, not yet published, from Croce and his collaborators (personal communication) who have sequenced an intact translocated c-*myc* gene from one of the so-called variant translocations (see legend to Fig. 2*d*) and have found no mutations either in the coding or in the untranslated exons. This implies that the state of exon 1 may after all be irrelevant to the deregulation of the translocated gene, although alterations to other conserved sequences 5′ to exon 1 cannot be ruled out.

Importance of context

Can any sense be made of the data so far? Yes, but only by disregarding for the time being the particulars of exon 1 and refocusing on the behaviour of the immunoglobulin locus, and the normal c-*myc* gene, in the course of normal B-cell differentiation. Both undergo sequential changes in transcriptional activity as the cell progresses from the proliferating lymphoblastoid stage to the terminally differentiated plasma cell in which transcriptional activation of the immunoglobulin locus reaches its peak. In lymphoblastoid cells, both the immunoglobulin locus and the c-*myc* gene are transcriptionally active; Burkitt's lymphomas represent a later stage of differentiation, in which the c-*myc* genes are probably normally silent and the activity of the immunoglobulin genes is increased. Mouse plasmacytoma cells, which have chromosomal translocations equivalent to those of Burkitt's cells, represent the terminally differentiated state, with immunoglobulin transcription at its height.

By making somatic cell hybrids between different combinations of these cell types, Croce and his associates have, in effect, been able to assay the regulatory signals acting on both translocated and untranslocated c-*myc* genes against each differentiated cell background. I have already discussed[1] the results of a series of experiments on the behaviour of untranslocated c-*myc* genes. The c-*myc* gene on the untranslocated chromosome 8 in Burkitt's cells is silent in the lymphoma cell but transcriptionally active in a lymphoblastoid cell background. The normal active c-*myc* genes of the lymphoblastoid cell, by contrast, are silenced when transferred to a mouse plasmacytoma cell. This presumably reflects the influence of stage-specific regulatory factors acting on the normal regulatory sequences of the c-*myc* gene.

In more recent experiments along the same lines[21], Croce and his collaborators have gone on to analyse the stage-specificity of transcriptional activation of the translocated c-*myc*, whose activity presumably reflects the normal regulatory influences on the immunoglobulin locus. Indeed, by choosing a Burkitt's cell line in which the translocation has broken both the c-*myc* gene and the immunoglobulin locus, they have been able to use the reciprocal translocations (Fig. 2*b* and *c*) to assay separately the regulatory elements of the two separated parts of the immunoglobulin locus on the separated parts of c-*myc* in two different backgrounds: lymphoblastoid and plasmacytoma.

The Burkitt's cell line that Croce and his co-workers used is ST486, in which there is a reciprocal translocation between chromosome 8, which contains the c-*myc* locus, and the immunoglobulin heavy-chain locus on chromosome 14. The break on chromosome 8 falls between exon 1 and exon 2 of c-*myc*, the two coding exons being transferred to the immunoglobulin locus on chromosome 14 (see Fig. 2*b*) while exon 1, left behind on chromosome 8 (Fig. 2*c*), is joined to a fragment of the immunoglobulin locus containing the one known tissue-specific regulatory sequence operating on the immunoglobulin genes — the enhancer sequence upstream of the constant-region segment (see ref. 1).

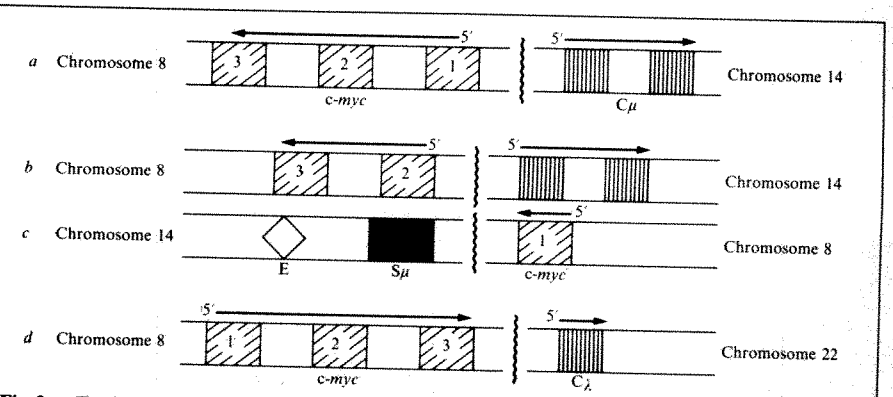


Fig. 2 *a*, Typical translocation with c-*myc* intact; *b*, typical translocation with c-*myc* separated from its exon 1; *c*, reciprocal of translocation shown in *b*. The typical translocation in Burkitt's lymphoma cells involves a reciprocal exchange between chromosome 8 and chromosome 14, resulting in the juxtaposition of the c-*myc* gene with the immunoglobulin heavy-chain constant-region locus C_μ, the two genes being in reverse transcriptional orientations (shown as 5′→). The distance of the chromosome 14 breakpoint from the constant-region locus is extremely variable; the breakpoint on chromosome 8 also varies, sometimes separating exon 1 of c-*myc* from the coding exons (*b* and *c*) and sometimes not (*a*). *d*, In the rare variant translocations, the translocation is between chromosome 8 and the immunoglobulin light-chain loci on chromosome 22, as shown here, or on chromosome 2. In these cases c-*myc* remains intact on chromosome 8 and is joined to the constant-region locus of one of the two light chains, tail to head, so that both are in the same transcriptional orientation.

One of the many earlier reverses in the attempt to understand c-*myc* activation in Burkitt's cells was the discovery that this transcriptional enhancer is usually separated from the immunoglobulin locus in the translocation, and thus cannot account for the transcriptional activation of the invading c-*myc* exons. Since the c-*myc* exons are activated notwithstanding, it became clear that there must be some other transcriptional activator operating on the immunoglobulin locus. Moreover, because some of the translocations place c-*myc* far away from the constant-region segment, it must operate over a very long range. Croce now believes he has indirect evidence of just such a long-range enhancer.

In hybrids between ST486 and lymphoblastoid cells, in which as a general rule the immunoglobulin genes of both parent cells are expressed, the translocated c-*myc* gene of the ST486 cell is silent — despite having lost its first exon, and thus being freed from any *myc* repressor. Apparently, this gene is not responding to the normal tissue-specific activation signals either for c-*myc* (normally active in lymphoblastoid cells) or for the immunoglobulin genes. Croce interprets this to mean that the putative factors responsible for transcriptional activation of the immunoglobulin locus in lymphomas are absent from the lymphoblastoid cell background; in other words, the activation of the putative long-range enhancer is strictly stage-specific.

The evidence for stage-specificity is strengthened by the behaviour of the reciprocally translocated exon 1 in plasmacytoma cells. In its original Burkitt's lymphoma background, the isolated exon 1 is transcribed, presumably under the influence of the identified immunoglobulin enhancer to which it is tail-to-tail. In plasmacytomas, however, it is silent, while translocated exons 2 and 3 of c-*myc* are transcribed. This is presumably because the enhancer is 'read' only at earlier stages of B-cell differentiation, the longer-range mechanism taking over at the plasma-cell stage and accounting for transcription of the translocated coding exons.

If I had to guess, I should say that exon 1 of the c-*myc* gene will turn out to be critical to the cell-cycle regulation of c-*myc* in its normal context; and that in Burkitt's cells, tissue-specific immunoglobulin gene activators simply override all local c-*myc* controls that might otherwise influence the translocated gene[16]. In short, from investigating c-*myc* regulation in lymphoma cells, we may learn more about the regulation of immunoglobulin genes than about the control of c-*myc* expression. □

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Archaeopteryx palaeontological myopia

SIR — Howgate[1] criticizes our interpretations[2,3] of the homologies and morphology of the *Archaeopteryx* manus by distorting and misrepresenting our viewpoints. Palaeontologists have identified the digits of theropods, *Archaeopteryx* and birds as digits 1-2-3 of the primitive reptilian hand whereas embryologists[4-7] have identified the bird digits as 2-3-4. Do the similarities observed by palaeontologists have greater weight than the evidence derived from ontogeny by embryologists?

The correspondence of the number of phalangeal elements in the manus of *Archaeopteryx* with the primitive reptilian formula has convinced palaeontologists that the digits of *Archaeopteryx* represent the digits 1-2-3. We stated that the digits of birds must be 2-3-4 based on embryological work[4,5]. If *Archaeopteryx* is a bird, it should have digits 2-3-4 and not 1-2-3 as suggested by Howgate[1].

Howgate[1] states that we[2,3] resurrected "a broken phalanx hypothesis" proposed by Heinroth[8] which states that there was a break in the proximal phalanx of topographic digit 3 and that we "arrived at a non-theropod phalangeal formula (2-3-3)". We cited Heinroth[8] because it was essential for a complete review. We[2] stated that "The break or joint in the first phalanx of the fourth digit appears immediately distal to the immobilized area". We again stated[3] (p.144) that "either a break occurred in the first phalanx (of the last digit) or if there are two phalanges a distortion of their orientation". We did not conclude that the phalangeal formula of *Archaeopteryx* was 2-3-3, but only stated the possibility of there being a break. Contrary to Howgate[1], only the Berlin and Maxberg specimens have the essential areas of the manus preserved as bone. Their state of preparation precludes a definitive statement of the phalangeal formula of *Archaeopteryx*.

In the left manus of the Berlin specimen there is a laterally directed flange on the first phalanx of the middle digit, directed to the junction of the supposed joint or break. Howgate implied that we[2] said that this flange "served to brace and strengthen the wing"[1]. Howgate[1] further states that "the 'flange' appears to be a secondary outgrowth due to injury or disease and not an original morphological structure". We did not state that this flange could serve to brace the wing since such a small structure cannot brace an entire wing. This functional misconception can only be attributed to Howgate. His illustration of the right manus demonstrates that this area is covered by matrix and a digit. Therefore how can he conclude the flange is an anomaly or a normal structure?

Attempting to make our explanation appear ludicrous, Howgate[1] implies that our "scenario" of the preservation of the fourth digit (its crossing under the third digit) was the result of *Archaeopteryx* "crossing its fingers hopefully as it plunges at high speed in the sea wing-tips first". We stated (as a scenario) that the break or joint in topographic digit 3 was caused by forces during stalling[9,10], impact upon the water, or as a result of preservation. Howgate[1] also stated that "all the specimens of *Archaeopteryx* with a well preserved manus have the second and third digits crossed". This is not the case since the Maxberg specimen[11] has a manus without this condition.

The important aspect of the homologies of the avian manus is the debate between the 1-2-3 and 2-3-4 hypotheses and the implication that different developmental patterns in digit reduction can produce similar morphologies.

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Evolutionary unease?

SIR — Peter Fellgett raises an interesting suggestion[1] that the study of cybernetic feedback loops throws light on the redundant (multiple copy) nature of functional genes and hence places the evolution of this phenomenon within the rubric of Darwinian natural selection. His proposition is that selection has favoured organisms in which gene redundancy not only protects them from mutation but also leads to enhanced prospects for survival in that 'a new [biological] program would run rather than crash'. He considers this system of 'selection for selectability' as more acceptable than so-called alternative *ad hoc* suggestions such as molecular drive, which he thinks should only be invoked as additional evolutionary mechanisms if the necessities were demonstrated; the impliction being that selection is our basic null hypothesis.

I have no objection to Fellgett's basic proposition, although I cannot understand his premise that the scientific necessity for alternative mechanisms depends on which side of the Darwinian fence they fall. Molecular drive[2] is based on several well-known processes of non-reciprocal exchange between redundant genes, all of which have the interesting and biologically important effect of continually replacing a family of genes with newly-arising mutant genes, throughout a sexual population. This mechanism of spreading a mutant gene, or parts of genes, can explain the widespread observation of clear differences between species in the way in which families have become replaced (homogenized). The phenomenon has been observed in families of both protein-coding and RNA producing genes and in non-functional DNA families, generally irrespective of the degree of redundancy or chromosomal dispersion of the families. Hence it is extraordinarily difficult to explain by selection alone the parallel homogenization of hundreds of families and the accidental gain and loss of complete subfamilies and families, across any one species, without invoking generally implausible *ad hoc* scenarios. Genetic turnover potentially constitutes an independent mechanism of population change with respect to both the *de novo* origin and the subsequent behaviour of redundant genes. Selection and drift can be expected to interact with this mechanism, depending on the gene family and population structure in question (see refs 2–9).

I am quite comfortable with Fellgett's idea that the origin of redundancy (and I would add the existence of nonreciprocal exchange processes within the redundant families) might be due to selection via cybernetic multiple feedback loops, that is, the processes underlying molecular drive have arisen via natural selection. There is nothing in evolution that cannot be explained by natural selection, if that is our starting point. However, this possibility would need to take into account why so many hundreds of qualitatively different genic and non-genic DNA families exist, why the degree of redundancy varies over several orders of magnitude both for the same family between species and for different families in the same species, why many families differentiate into multiple subfamilies, and why there are continual rounds of family homogenization by variant genes or parts of genes across a species. An unsubstantiated appeal to a seeming Darwinian "selection for selectability" based on cybernetic theory is not a sufficient justification for discounting the necessity for an additional evolutionary mechanism which is based on known DNA turnover mechanisms within the genome. That molecular drive is non-Darwinian is surely irrelevant.

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The active site of acidic aluminosilicate catalysts

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The availability of solid acidic catalysts based on zeolite ZSM-5 make possible observations on the nature and the absolute rate behaviour of the individual protonic sites. Tetrahedral aluminium atoms are the highly reactive ingredients, even at levels of parts per million, or less. Turnover numbers for several hydrocarbon reactions equal and exceed familiar enzymatic turnover values. Intensive catalytic activities can result from aluminium levels likely to be ignored by the experimenter.

THE acid catalytic activity of aluminosilicates is usually assumed to reside in sites involving tetrahedral aluminium atoms in substitutional positions in the framework of silica; the negatively charged aluminium sites are associated with cations which, in the case of acid catalysts, are protons. It has, however, been difficult to identify and count the number of active sites, and thus to determine quantitatively the turnover rate of which these sites are capable. With the availability of zeolitic catalyst, and particularly of ZSM-5 catalysts, such a quantitative description is now possible.

For many decades, acidic clays and amorphous silica–alumina compositions have been used to catalyse the cracking of carbon–carbon bonds, for alkene isomerization and polymerization, aromatic alkylation with alkenes or alcohols, transalkylation, and other acid-catalysed reactions. The cracking of alkanic bonds requires the highest activity; alkene transformations require lower catalytic strength and/or lower temperature.

Activity of aluminosilicates

A convenient measure of activity is based on the alpha (α)-test[1,2] which has been refined[3]. It consists of measuring the first-order rate constant for the cracking of *n*-hexane at 538 °C at 100 mm Hg. For comparing relative activities, $\alpha = 1$ corresponds to the activity of a high surface area (420 $m^2 g^{-1}$) amorphous silica–alumina catalyst (10 wt% Al_2O_3 content), which has a first-order rate constant for *n*-hexane cracking of $k = 0.067\ s^{-1}$. This corresponds to a reaction rate ($dn/dt = kc$) of 1.3×10^{-7} mol $s^{-1} g^{-1}$ of catalyst characteristic of highly active catalyst particles in commercial petroleum cracking.

By definition, k is the inverse of a residence time (for 63% conversion). Previously[2], we have used the superficial residence time, based on vapour flow rate into the reactor measured at room temperature, and the bulk catalyst volume; this gave a rate constant of $0.016\ s^{-1}$. This is convenient when relative activities (α) are to be determined for practical reactor operations. To describe an absolute rate, we use the more accurate value based on vapour flow in reaction conditions (538 °C) and on unit weight of catalyst.

As the above catalyst contains about 0.002 mol of Al g^{-1}, the turnover rate above corresponds to only 4×10^{-3} molecules min^{-1} per atom of total aluminium in the sample. However, the fraction of the Al atoms which are both accessible and in the proper chemical condition (tetrahedral substitution in silica structure) is unknown.

With the zeolites as catalytic solids, we have progressed from solids with a, usually unknown, number of active sites available on the geometric particle surface to an essentially 'transparent' medium: all intra-crystalline sites can be available for molecular contact.

With zeolite Y in the hydrogen (protonic) form, α activities of between 1,000 and 5,000 and higher were observed[1,2]. This implies a turnover capability of the order of 2–10 molecules min^{-1} per Al atom. However, the equivalence and degree of participation of all Al atoms was not clear in view of the high aluminium atom density in this zeolite, approximately one Al atom for two Si atoms, and the ease with which aluminium can undergo hydrolysis and removal from the framework.

Physical chemistry of ZSM-5

In contrast to zeolites previously used as catalysts, the aluminium atom concentration in zeolite ZSM-5 is very low and continuously variable over many orders of magnitude, as illustrated by Fig. 1. The representation of aluminium concentration when expressed in terms of the SiO_2/Al_2O_3 ratio traditionally adopted to characterize zeolite mineral structures, demonstrates the variability and unique diluteness of Al-atom concentration achievable for ZSM-5.

The factor of most physical chemical significance is that, in ZSM-5, Al atoms are no longer structure-determining constituents, that is, they no longer contribute a major portion of the framework structure[3].

The average distance between aluminium sites is equivalent to several to many atomic diameters, and their distribution should approach randomness. It has been observed[4] that a random model (subject only to the proviso of Loewenstein's rule) may be applicable to a zeolite at Si/Al ratios greater than a value of 2.0.

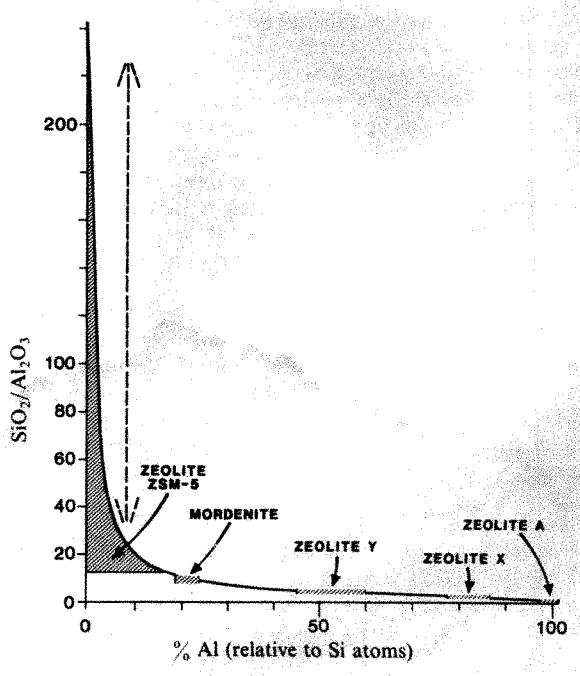


Fig. 1 Al atom concentration and SiO_2/Al_2O_3 ratio for various zeolites.

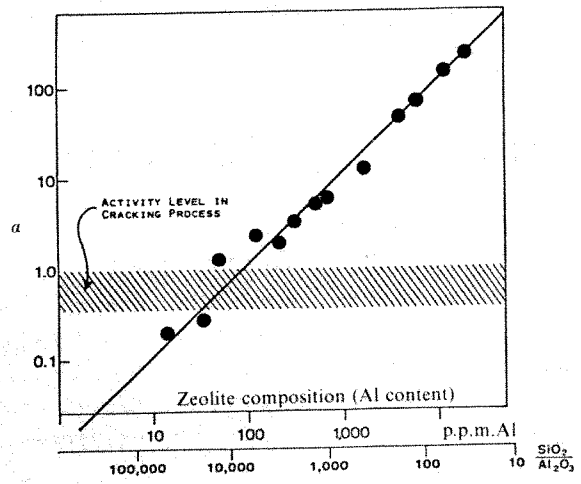


Fig. 2 The hexane cracking activity plotted against the aluminium content in HZSM-5. Shaded band indicates activities near $\alpha \simeq 1$.

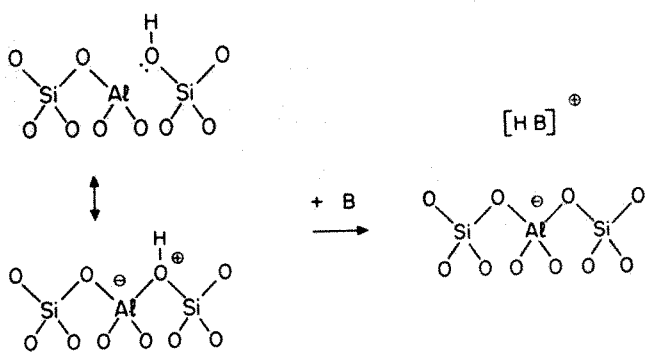


Fig. 3 The Brønsted acid site in HZSM-5 and its interaction with a base.

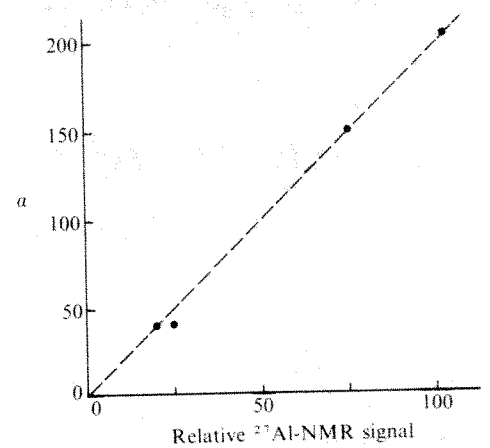


Fig. 4 The activity of HZSM-5 plotted against the tetrahedral aluminium NMR signal.

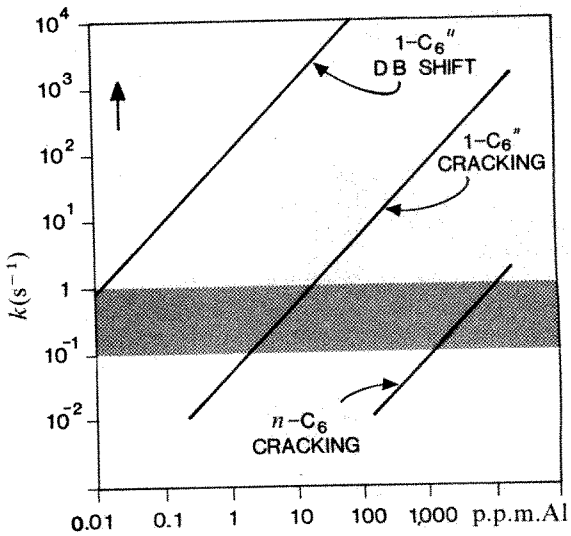


Fig. 5 Comparison of rate constants at 454 °C, of hexane cracking, hexene cracking, and hexene double-bond (DB) shift (after ref. 18).

Elementary statistics indicate that in an assembly of 96 T-atoms (Si or Al), which define a unit cell of the ZSM-5 structure, a fraction of approximately 14% of all unit cells would have no Al atoms, when the average Si/Al ratio R of the sample is 48, according to $f=[1-1/(R+1)]^{96}$. Conversely, for a ZSM-5 zeolite of a higher average Si/Al ratio of, say, 120, about 20% of its unit cells can be expected to have a Si/Al ratio of <50.

These calculations apply rigorously to samples with a macroscopically homogeneous aluminium distribution, both within the individual crystallites and within the sample. Some authors[5–7] have reported Al gradients in their samples. For such samples, with average Si/Al value R, statistics will produce some deviation of Al frequency per unit cell entity in both directions.

In all cases, it will become apparent from the catalytic data that the Al atoms in ZSM-5 occur at sufficiently low concentration to be analogous to a dilute solution. There is substantial chemical uniformity of sites. (This does not rule out topologically different locations in the ZSM-5 framework, as they involve characteristic distances beyond the range of localized chemical forces.)

C–C bond cracking activity per site

The α activity is proportional to Al contents and extrapolates to zero for aluminium concentration tending to zero[3]. This dependence has now been extended over nearly four orders of magnitude of both activity and Al concentration, down to nearly 20 p.p.m. of Al, as seen in Fig. 2. For orientation, activities near and below $\alpha \sim 1$ correspond to levels actually used by industrial processes.

The active Brønsted acid site in a hydrogen zeolite and its interaction with a base or substrate is depicted in Fig. 3. Magic-angle spinning (MAS)–NMR data have shown[8] that in the dehydrated state aluminium is in a tetrahedral environment which is asymmetric, in agreement with Fig. 3 (left-hand side). On interaction with an added base, B, such as water[8] or ammonia[9], the aluminium is in a highly symmetrical tetrahedral coordination[8], as indicated in Fig. 3 (right-hand side).

A meaningful quantitative correlation of activity with total aluminium content can be expected only when all Al atoms are in tetrahedral framework positions. Hydrolysis to give extra-lattice Al is frequently observed in zeolites with low Si/Al ratio such as zeolite Y[10]. In HZSM-5, this can be readily prevented by preparing the sample in conditions that avoid undue exposure to heat and water vapour[3], as has been pointed out elsewhere[11,12]. Also, thorough replacement of sodium ion must be assured, as its concentration must be only a small fraction of the already small Al concentration.

The complete proportionality of activity and number (concentration) of Al atoms is consistent with the homogeneity of the active sites achieved in this state of dilute solution.

This and the existence and stability of a single protonic Al site in HZSM-5 is also supported by and consistent with the following independent observations:

(1) Examination of the Al atoms using high-resolution MAS–NMR techniques[13] has demonstrated that all aluminium incorporated during synthesis of ZSM-5 zeolite, even at the lowest level of incorporation in the synthesis, is tetrahedral in structure.

(2) In carefully prepared samples there is a single hydroxyl band, independent of the Si/Al ratio, centred at 3,603 cm^{-1} in

the IR spectrum assigned to Brønsted acid sites[12], and the intensity is proportional to Al content. A second IR hydroxyl band at about 3,720 cm^{-1} is due to crystal terminating OH groups or to silica gel impurities[12,14].

(3) The ammonium form can be quantitatively reconstituted and no Lewis-bound pyridine can be found by IR[12], that is, all Al atoms in the zeolite framework are in tetrahedral coordination.

(4) Measurements of the ion exchange capacities of a series of HZSM-5 samples with various Si/Al ratios show a 1:1 molar ratio of the exchanged cation, caesium, to the aluminium content[3].

(5) The temperature-programmed desorption (TPD) spectrum of ammonium-exchanged ZSM-5 shows a single high-energy desorption band with T_{max} at 400 °C as observed by Kerr and Mikovsky, and others[14]. Kerr and Mikovsky (unpublished data) have shown that it is not composed of a multitude of bands resulting from ammonia elimination from acid sites of different acidity, as is found with HY zeolite[15]. The molar amount of ammonia eliminated is equal to that of the chemically determined total aluminium concentration for a series of HZSM-5 samples[9]. After exposure to excess ammonia vapour, the ammonium form desorbs ammonia from two additional lower energy states, giving rise to three desorption maxima, that are sometimes erroneously thought to indicate the presence of three types of acid site[16,17].

We can now demonstrate in Fig. 4 the proportionality of α activity to the tetrahedral Al concentration, as determined by MAS–NMR[9]. Clearly, the tetrahedral Al atoms (Fig. 3) and their associated protons constitute the active site.

With such quantitative data for the number of active sites available, absolute molecular turnover rates and numbers can now be determined.

The absolute turnover rate for *n*-hexane cracking at the standard test conditions (538 °C, 100 torr *n*-hexane pressure) is calculated to be about 2.8 molecules min^{-1} per atom of Al, for the entire Al concentration range.

We find that the reaction rate is indistinguishable from first order for the entire pressure range 10–760 torr. Therefore, the fraction of occupied Al sites at the pressure of measurement (100 torr) must be <1% of all Al sites. It follows that the turnover rate per occupied active site (the turnover number) is greater than 300 molecules min^{-1} per active Al site.

Acid-catalysed turnover rates

The intrinsic rate constants for many other acid-catalysed reactions are proportional to the α of the ZSM-5 catalysts, and therefore to the number of Al atoms; they include the rates for toluene disproportionation, xylene isomerization, alkene conversion and methanol conversion[18]. These rates are all greater than the alkane cracking rate.

Figure 5 compares the reaction rates we have observed for *n*-hexane cracking, 1-hexene cracking, and 1-hexene double bond isomerization. Each absolute rate was found to be proportional to tetrahedral aluminium concentration, as shown by the parallel lines. The alkene cracking rate is some 800 times the alkane cracking rate, and the alkene isomerization is some 1,300 times the alkene cracking rate.

Many commercial processes occur at a liquid hourly space velocity (LHSV) of 1–10, that is, they occur with a rate constant $k \approx 0.1$–$1\ s^{-1}$. Thus, the site concentration needed for achieving such technically relevant reaction rates—a magnitude indicated by the shaded area in Fig. 5—can be below 1,000 p.p.m. Al for alkane conversion, and as low as a fraction of a p.p.m. to 100 p.p.m. for various other conversions such as those involving alkenes. Hence 'discoveries' of significant but unexpected catalytic activity on siliceous zeolite compositions can occur due to ignorance of the role of unanalysed amounts of lattice aluminium.

Table 1 The magnitudes of the turnover values for ZSM-5 at 454 °C, 100 torr

	Turnover rate (molecules min^{-1} per Al site)	Turnover no. (molecules min^{-1} per active Al site)
n-hexane cracking	0.37	>37
1-hexene cracking	290	>29,000
1-hexene double-bond isom.	370,000	$\geq 4 \times 10^7$

From data of Lago and Haag; see ref. 18.

Table 1 presents the magnitudes of the turnover figures at 454 °C, expressed in the units (min) characteristic of enzymology. (These magnitudes are large and not dissimilar from those encountered in enzyme catalysis.)

Experimental procedures

The samples of HZSM-5 were prepared[19] and pretreated[3] according to published procedures. Total aluminium was determined by digesting the zeolite in a HF/H_2SO_4 mixture, evaporating to dryness, dissolving the residue in hydrochloric acid and, after addition of potassium chloride as an ionization and interference suppressant, analysing by atomic absorption spectrometry. The accuracy of the Al analysis depends on the concentration in the original sample with the following uncertainties: for 1,000, 100 and 25 p.p.m. Al—5%, 10% and 25%, respectively.

The concentration of framework tetrahedral aluminium was determined quantitatively by ^{27}Al MAS–NMR using a JEOL FX-200 Fourier transform spectrometer. Details of the technique will be published elsewhere[9].

Conclusions

It has been experimentally demonstrated that the catalytic site for acid catalysis on aluminosilicate catalyst is quantitatively identifiable with a protonated tetrahedral aluminium atom in the SiO_2 framework.

The number of sites and associated acid activities have been determined, using ZSM-5 zeolites of varying Al concentration, from which absolute turnover rates and numbers are now available. These resemble in magnitude the enzymatic turnover numbers.

Careful control and quantitative knowledge of minute Al concentrations is essential in experimentation and interpretation of catalytic observations on ZSM-5 structures and other zeolites. If ignored, the results are likely to be erroneously attributed to other phenomena or components.

If we assume that the active sites in standard amorphous silica–alumina catalysts are similar in nature and specific activity to those in ZSM-5, then the above data indicate that only about 1 out of 1,000 of the analysed Al atom was effectively involved in such catalysts.

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Effect of somatic mutation within translocated c-*myc* genes in Burkitt's lymphoma

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Our previous studies of a translocated c-myc *gene in the Raji Burkitt's lymphoma cell showed somatic mutations in exons 1 and 2. We have extended these observations to two other translocated* c-myc *genes and find a common occurrence of mutation in the noncoding exon 1. We also found that in Raji cells, unlike other Burkitt's lymphoma cell lines, the normal allele of the* c-myc *gene is transcribed as well as the translocated gene. These results support a model in which* c-myc *oncogene activation in Burkitt's lymphoma occurs by disruption of a normal transcriptional control mechanism in which the* c-myc *protein is itself involved.*

BOTH human Burkitt's lymphoma (BL) and mouse myeloma cells display specific chromosomal translocations involving immunoglobulin gene loci and the c-*myc* proto-oncogene[1–7]. In Burkitt's lymphoma there are three types of translocation involving the c-*myc* gene on the long arm of chromosome 8 (8q24). These frequently involve the immunoglobulin heavy (H)-chain locus (14q32) and less frequently, in the so-called variant Burkitt's lymphoma, involve either the κ light (L)-chain locus (2p12) or the λ L-chain locus (22q11)[8]. Somatic cell genetics[9] and *in situ* hybridization studies[10] established that in t(8; 14) the c-*myc* gene is carried into the $14q^+$ aberrant chromosome where it becomes associated with the H-chain constant (C)-region genes in the opposite orientation for transcription[6,7,11,12]. Frequently this translocation to chromosome 14 does not affect the three-exon structure of the c-*myc* gene nor does the breakpoint occur near to the c-*myc* gene transcriptional promoter[6,12]. A more dramatic variation in the breakpoint relative to the c-*myc* gene has been reported in the variant BL translocations where it occurs downstream (3′) of this gene[10,13–16]. In contrast, the analogous translocation of the mouse c-*myc* gene and the *Igh* locus very frequently results in complete or partial loss of the c-*myc* gene exon 1 (refs 2, 4, 17).

In view of this variability in the translocation breakpoint with respect to the c-*myc* gene in Burkitt's lymphoma and mouse myelomas, the possibility must be considered that, assuming the translocation activates the oncogenic potential of the c-*myc* gene, controlling elements normally involved in immunoglobulin gene transcription take control of the translocated c-*myc* gene. The possibility of higher levels of transcription resulting from translocation does not seem to be the whole answer because we were unable to demonstrate elevated levels of c-*myc* messenger RNA (mRNA) transcription in BL cell lines compared with lymphoblastoid or other tumorigenic cell lines[6]. Furthermore, a clearly demonstrable enhancer element near the human C_μ gene does not remain on the $14q^+$ chromosome in many cases of t(8; 14)[18] and therefore cannot control this gene transcription in these cases. As no obvious general effect could be observed on the c-*myc* gene in Burkitt's lymphoma, we investigated the possibility that alterations in the existing elements for controlling the c-*myc* gene are important rather than the acquisition of new elements after translocation. This focused our attention on exon 1 of the c-*myc* gene, which has no assigned function, being transcribed but not translated into protein[6,19,20] although it has been suggested to have a regulatory role[21]. Recently, we described the nucleotide sequence of the translocated c-*myc* gene from Raji cells t(8; 14) and found substantial alterations in the sequence (both base changes and deletions) of the first two exons[22]. The changes observed in Raji exon 2 would result in a substantially altered c-*myc* protein product, but no somatic mutations were reported to be present in another translocated c-*myc* gene sequenced recently[7]. These data suggested the possibility that changes in the sequence of exon 1, rather than coding-region segments, may be important in the activation of the c-*myc* gene. We now report the sequence of the 5′ end of a Daudi c-*myc* cDNA clone[6] which must derive from the translocated c-*myc* gene as only this allele of c-*myc* is active in Burkitt's lymphoma[12,23]. This sequence differs from the normal exon 1 by 7% of base substitutions. Furthermore, the sequence of the translocated c-*myc* gene exon 1 from the variant BL cell line LY67 [t(8; 22)] was also found to contain base substitutions. In addition, we have observed that the normal c-*myc* gene is expressed in Raji cells (which possess a substantially altered translocated c-*myc* gene-coding segment), unlike the analogous gene in the majority of BL cell lines. This result supports a transcriptional control mechanism using, directly or indirectly, the c-*myc* gene product.

Variable translocation point

In the cell lines studied here [Daudi, t(8; 14); JI and LY91, t(2; 8); MAKU and LY67, t(8; 22)], we were unable to detect rearrangements of the c-*myc* gene using an exon 2 probe (M13JI1.5Sac)[6] in Southern filter hybridization with *Eco*RI or *Bam*HI-digested DNA (data not shown). Several c-*myc*-containing λ phage clones (see Fig. 1), were isolated from phage libraries prepared from BL DNA and a detailed restriction map of the c-*myc* locus was constructed covering approximately 8 kilobases (kb) upstream of exon 1 and 14 kb downstream of exon 3. Two subclones were prepared (pUCJI5R4 and pUCJI7BS8) (Fig. 1) and used in filter hybridizations with either *Bgl*II-digested genomic DNAs (pUCJI5R4) or *Sac*I-digested DNAs (pUCJI7BS8). The choice of these two restriction enzymes allows us to overlap the DNA segments detected by the exon 2 hybridization probe and therefore assay for possible DNA rearrangements which have occurred on the flanking sides of the probes. We only detected unrearranged restriction fragments with either probe (6.5-kb *Bgl*II fragment and 7.7-kb *Sac*I fragment) as evidenced by the similarity between all of the DNAs compared (Fig. 1). As we know that the translocation point is upstream of the c-*myc* gene in Daudi cells[11], these hybridization data place the translocation point at least 12 kb upstream of this c-*myc* gene, in agreement with results reported by others[11,12]. Conversely, as the breakpoint is on the 3′ side of the c-*myc* gene in variant BL cells, the lack of rearrangement present in *Sac*I-digested DNA from JI, LY91, MAKU and LY67 shows the translocation point to be >14 kb downstream of the c-*myc* gene in these cells. Due to the remarkable variability between the c-*myc* gene and the translocation breakpoint in Burkitt's lymphoma[24] (see Fig. 1), none of the clones obtained from the

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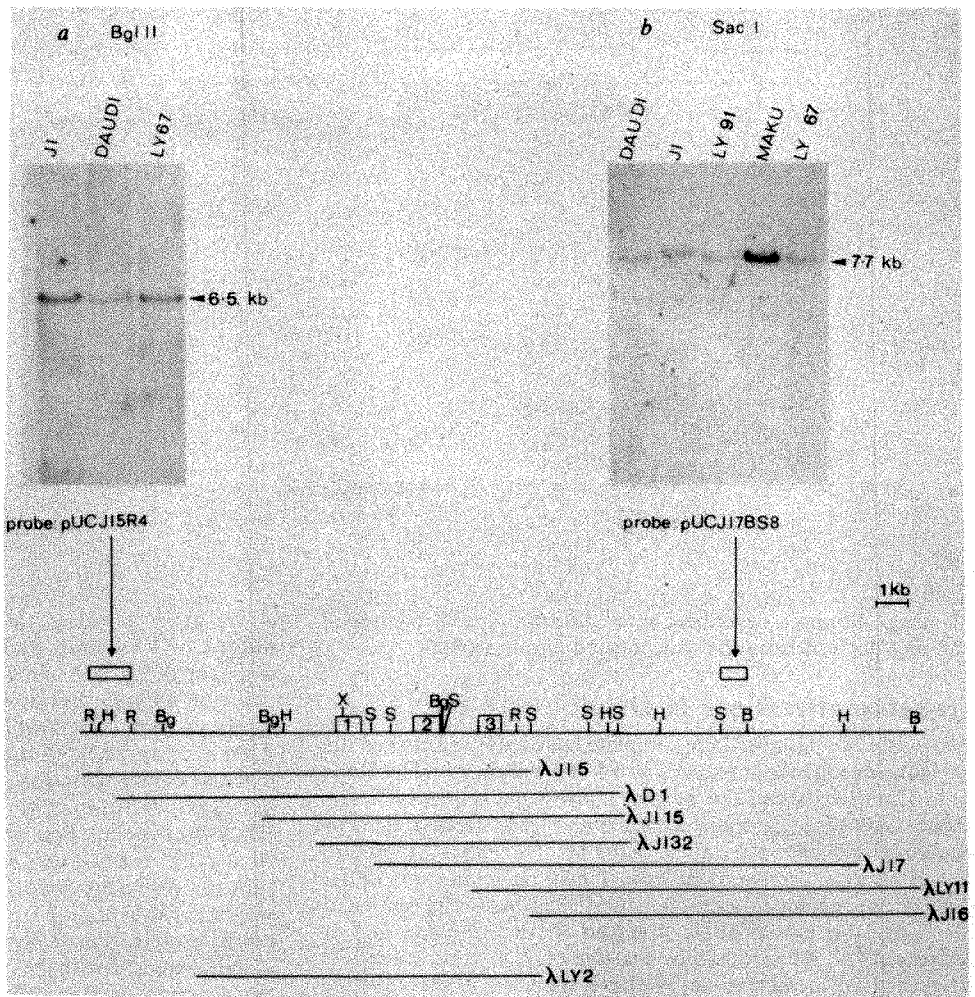


Fig. 1 Filter hybridization of Burkitt's lymphoma DNA using c-*myc* flanking region probes. The restriction map shown for the c-*myc* locus was generated from the λ phage derived from genomic libraries of JI and LY67 DNA (cloned in λ2001; ref. 34) and Daudi DNA (cloned in λ1059; ref. 34). The extent of each of these clones is indicated below the map (λJI7 has been described previously[22]). Two subclones used in this study, cloned in the pUC8 vector, were pUCJI5R4 containing a 1.2-kb *Eco*RI fragment from the 5′ end of the region and pUCJI7B58 containing a 0.8-kb *Bam*HI–*Sac*I fragment. These probes were nick-translated and used in Southern filter hybridizations[35] of *Bgl*II-digested DNA (pUCJI5R4, *a*) or *Sac*I-digested DNA (pUCJI7B58, *b*). Hybridization conditions were 2×SSC, 10% dextran sulphate, 0.2% polyvinylpyrrolidone (PVP), bovine serum albumin (BSA) and Ficoll[36], 0.1% SDS, 50 μg ml^{-1} fragmented salmon DNA containing 10^6 c.p.m. ml^{-1} probe (specific activity ~10^8 c.p.m. μg^{-1}); after hybridization, filters were washed with 0.1× SSC, 0.1% SDS and autoradiographed at −70 °C with prefogged film[37]. Restriction fragment sizes were determined by comparison with λ phage DNA cut with *Hin*dIII. R, *Eco*RI; H, *Hin*dIII; Bg, *Bgl*II; X, *Xho*I; S, *Sac*I; B, *Bam*HI; *Bgl*II sites are not complete.

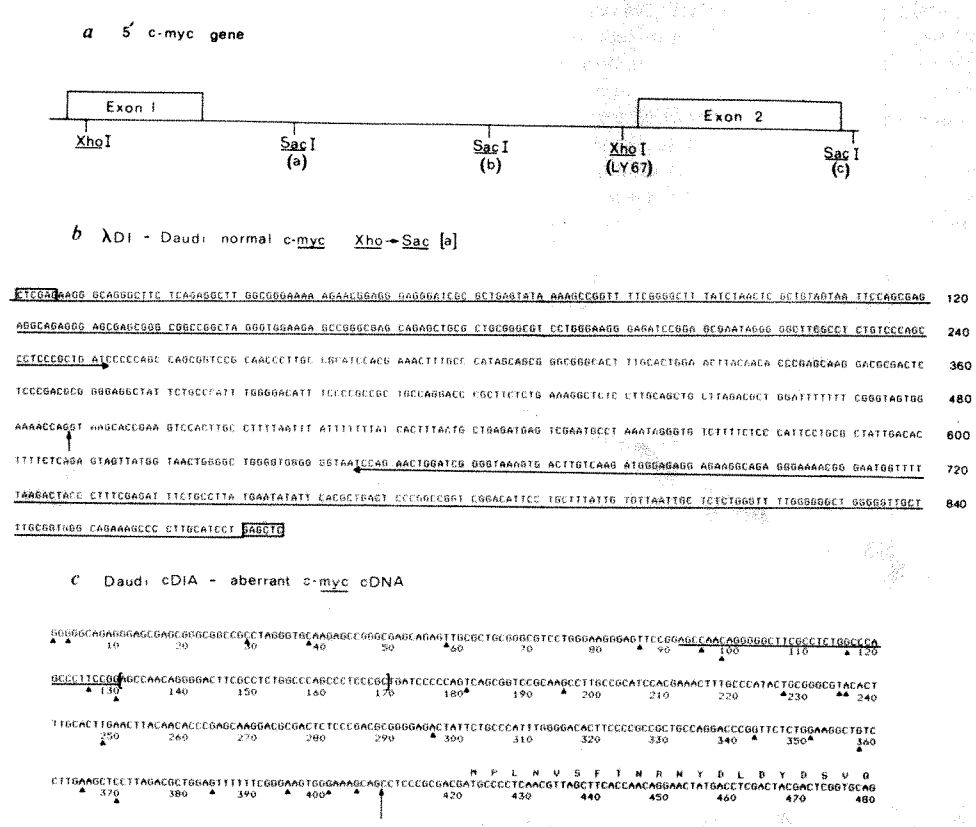


Fig. 2 Nucleotide sequence of exon 1 from the Daudi c-*myc* gene. *a*, Partial restriction map of c-*myc* exons 1 and 2. The *Sac*I site indicated as *Sac*I(a) is absent from clone λLY2 and the *Xho*I site near exon 2 is unique to λLY2. *b*, Exon 1 sequence of λD1 Daudi c-*myc* gene. *Xho*I and *Sac*I sites at the ends of the sequenced fragment are boxed. The vertical arrow indicates the RNA splicing signal. The underlined regions indicate sequences obtained from the analogous region of λJI5 and λJI15. Nucleotide sequences are obtained from random sonicated fragments subcloned in M13mp8 followed by sequencing by dideoxy chain termination methods[38]. *c*, 5′ end sequences of Daudi cDNA clone, pUC cDIA. The sequence variations observed for pUC cDIA[22] are indicated by small arrowheads (the 5′ G residues may have been introduced by cloning procedures); the large vertical arrow indicates the RNA splice site. The underlined region is a duplicated segment present in the adjacent bracketed section.

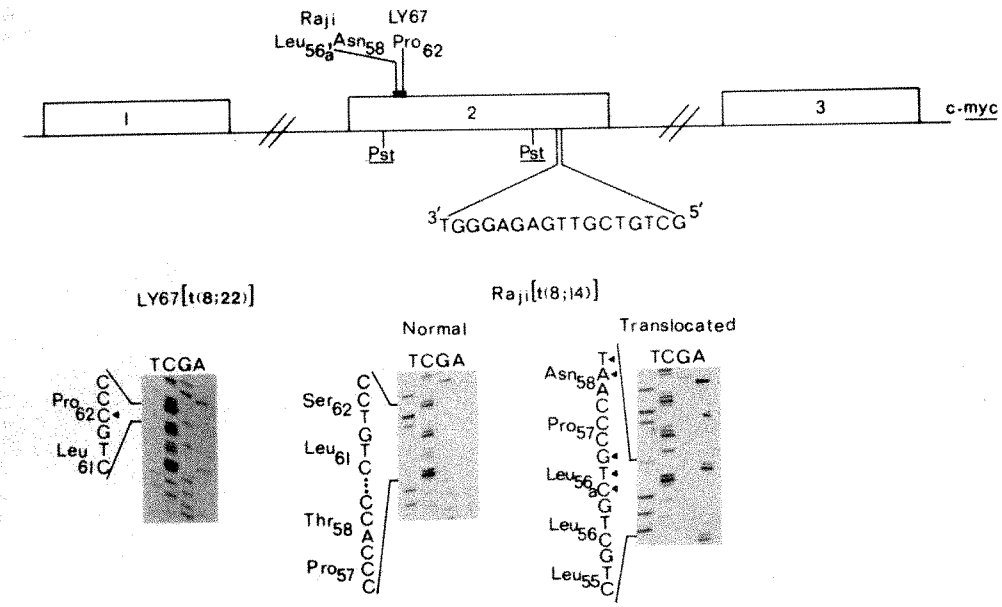


Fig. 3 cDNA cloning strategies and sequences from LY67 and Raji clones. A synthetic oligonucleotide (sequence shown beneath a diagrammatic representation of the c-*myc* genomic gene arrangement) was used to prepare double-stranded cDNA as described previously[39] from poly(A)-selected RNA obtained from post-nuclear supernatants. After digestion with *Pst*I, fragments were cloned into *Pst*I-digested M13mp8 and screened[40] for the presence of c-*myc* exon 2 sequences using a radioactively labelled *Sac*I fragment isolated from the clone M13JI71.5Sac[22]. Positive clones were directly sequenced by dideoxy chain termination procedures. The c-*myc* gene diagram indicates the position of amino acid substitutions found in LY67 and Raji genes as well as the *Pst*I sites used in the cloning, and representative areas of sequence are shown from the cDNA clones together with the base changes (indicated by arrows) present in altered versions.

BL phage libraries described here could be directly identified as being derived from the translocated chromosome.

Sequence alterations in Daudi c-*myc* cDNA

Previously, we described the complete concordance between the nucleotide sequence of exons 2 and 3 of c-*myc* in a cDNA clone from Daudi cells and the normal c-*myc* gene sequence[22]. The observation that the translocated c-*myc* gene from Raji cells contained sequence alterations in the first two exons[22] therefore suggested that alterations in exon 1 may be important (rather than coding region alterations). As it has been demonstrated that only the c-*myc* allele involved in the translocation is expressed[12,23], we can assume that the cDNA clone is derived from the translocated allele. We have, therefore, sequenced the exon 1 region of the Daudi cDNA clone and found that it contains multiple base differences compared with the sequence of exon 1 derived from the normal c-*myc* gene isolated from a phage library of Daudi DNA (included in a *Xho*I–*Sac*I fragment; see Fig. 2*a*). The latter sequence agrees with the exon 1 sequence of other normal c-*myc* genes[7,22,25] and those regions underlined in Fig. 2*b* were also sequenced in c-*myc* clones from JI phage clones and found to contain no sequence differences. This suggests that we are not merely detecting polymorphism of the human c-*myc* exon 1 sequence. The distribution of base alterations in the Daudi cDNA exon 1 is indicated in Fig. 2*c*, as well as the presence of a 35-nucleotide duplicated region (starting at residue 92). The overall difference between the cDNA and normal exon 1 sequences is 7.3% (excluding the duplication) but no obvious significant pattern of these base changes is apparent from this sequence. These data argue, however, that the differences observed in the Daudi cDNA exon 1 sequence result from mutations introduced into this region during the translocation event.

Markers for translocated c-*myc*

As discussed for the data in Fig. 1, those variant BL cell lines available for study did not show obvious c-*myc* gene rearrangement when analysed by filter hybridization and therefore did not provide us with phage clones which were obviously derived from the translocated chromosome. We therefore adopted an indirect strategy for the identification of the translocated c-*myc* gene which relies on the critical observation that the normal c-*myc* allele is transcriptionally silent in Burkitt's lymphoma[12,23]. Thus, the c-*myc* mRNA in BL cells should come from the translocated allele, and it follows that cDNA clones made from this mRNA will represent the sequence of this translocated c-*myc* gene. We have, therefore, prepared cDNA clones from mRNA of LY67 and Raji cells using the procedure indicated at the top of Fig. 3. A 17-residue long oligonucleotide (designated common oligonucleotide—see Fig. 5) was used to prime cDNA synthesis from a site near the 3′ end of exon 2 in the c-*myc* gene. After making the DNA double-stranded with DNA polymerase, it was cleaved with *Pst*I (for which sites occur in exon 2 as indicated) and cloned in the vector M13mp8 (ref. 26) for direct DNA sequencing. Six independent cDNA clones were made from LY67 mRNA and each of these clones showed a T to C substitution in the codon for amino acid 62. This substitution would yield a proline instead of serine at this position in the c-*myc* protein; Fig. 3 compares the relevant area of the LY67 cDNA sequence with the normal sequence derived from a cDNA clone present in Raji cells (this clone is discussed below).

The presence of a proline codon substitution in the LY67 cDNA provides a sequence marker for the translocated c-*myc* gene in this cell line. By comparing this sequence with genomic clones derived from LY67 DNA, we found that the clone λLY2 (see Fig. 1) contained this change and therefore conclude that this genomic DNA insert was representative of the c-*myc* gene involved in the translocation in this cell. An indication of possible sequence differences at the 5′ end of the genomic insert within λLY2 was given by two restriction enzyme site polymorphisms found in the intervening sequence between exons 1 and 2. These are the acquisition of a new *Xho*I site (resulting from an A→G transition) 49 bases from the ATG codon (shown in Fig. 2*a* and Fig. 4) and the loss of a *Sac*I site shown as *Sac*I(a) in Fig. 2*a*.

The strategy for sequencing the relevant regions of λLY2 was to subclone the 2-kb *Xho*I fragment (containing exon 1) in pUC8 and the 1.5-kb *Sac*I fragment (containing exon 2) into M13mp10, followed by fragment isolation for generation of randomly overlapping clones. A partial sequence of the segment from the 5′ *Xho*I site to the *Sac*I(c) site (see Fig. 2*a*) of the λLY2 insert is shown in Fig. 4. The only nucleotide alteration, compared with normal c-*myc* gene sequences, found in exon 2 is the single base change identified in the cDNA clones. Further nucleotide substitutions begin to appear in the intervening sequencing between exons 1 and 2, and multiple differences exist within exon 1 itself. Interestingly, in this case the base substitutions are confined to the 3′ half of exon 1, in marked contrast to the overall spread of changes found in exon 1 of Daudi and Raji cells. Furthermore, sequence data of the promoter region of the LY67 translocated c-*myc* gene revealed no base substitutions compared with the normal sequence (unpublished results). A possible significant feature of these findings is that in three cases of translocated

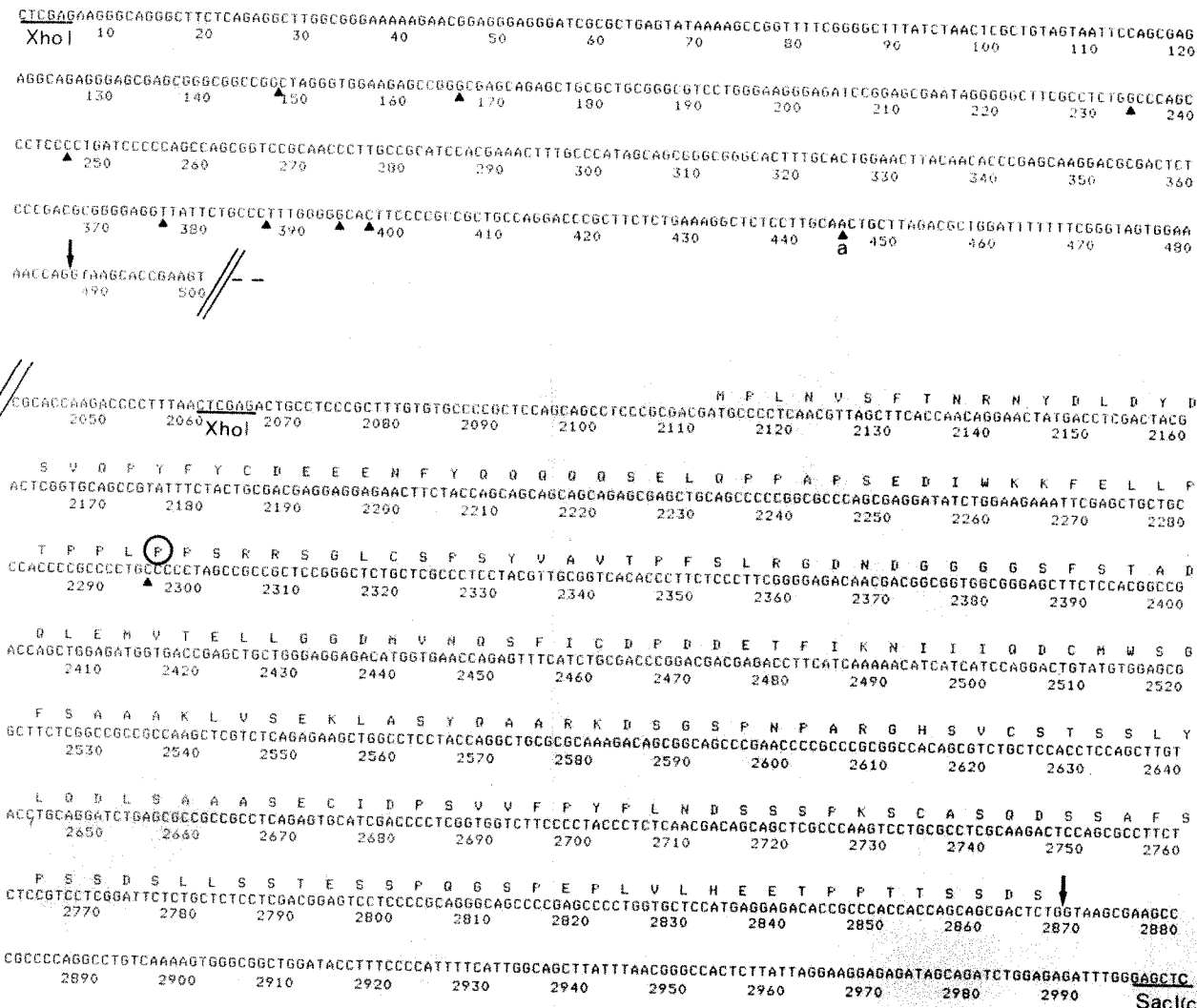


Fig. 4 Partial nucleotide sequence of the aberrant c-*myc* gene derived from λLY2. The nucleotide sequence is shown from the *Xho*I site to the *Sac*I(c) site (Fig. 2) of λLY2. Sequence data were obtained from random sonicated subclones in M13mp8 or subclones derived from either *Pvu*II, *Taq*I or *Alu*I digestion of a clone containing the 2-kb *Xho*I fragment (indicated in Fig. 2) in pUC8 (pUCLYXh16). Base substitutions within exon 1, compared with normal c-*myc* (Fig. 2), are indicated by small arrowheads and deletions are indicated by arrowheads between bases (deletions a and b are 2- and 3-base pair deletions respectively; the former results in the destruction of a *Pvu*II site). The proline substitution in exon 2 is circled. Restriction sites are as indicated in Fig. 2 (the *Sac*I(a) site is absent from λLY2 and the *Xho*I site at position 2,061 is unique to λLY2). The RNA splice sites are marked by large arrows.

c-*myc* genes (those of Raji, Daudi and LY67 cells), exon 1 is altered compared with the normal sequence (see below).

Normal c-*myc* is transcribed in Raji cells

Usually, the unrearranged c-*myc* gene is transcriptionally silent in Burkitt's lymphoma[2,23], strongly implying a direct transcriptional control of the c-*myc* gene which is, however, lost from the translocated allele. As this effect seems to be related to the product of the c-*myc* gene, it would be of considerable interest to study the effect of mutants in the c-*myc* protein on c-*myc* gene transcription. Potentially, the product of the translocated c-*myc* gene in Raji cells represents such a situation as it has been shown that exon 2 (the first coding exon) contains several codon substitutions. Therefore, we have prepared cDNA clones from Raji mRNA to investigate the possibility that the normal c-*myc* allele is active in the presence of an altered gene product. Two cDNA clones were prepared (see Fig. 3) and the sequences revealed that one of these clones derived from the translocated c-*myc* gene, as it contained the characteristic base changes present in this gene, while the other cDNA clone derived from the normal allele. Figure 3 includes relevant sequences from these clones, showing the regions of interest. The analysis of only two cDNA clones from Raji does not allow any real estimate of the relative proportions of transcription (or of translation) from the respective c-*myc* genes. We therefore undertook to estimate the relative level of c-*myc* gene transcription in Raji cells using oligonucleotides which either detect both the normal and translocated c-*myc* mRNA (common oligonucleotide) or detect only the translocated c-*myc* mRNA (Raji-specific oligonucleotide) in Northern mRNA filter hybridizations. Figure 5 shows the results of this type of experiment; Fig. 5*a* illustrates the 17-long oligonucleotides used and their corresponding locations in exon 2 of c-*myc*. The common oligonucleotide is the one used for the cDNA cloning experiment and the Raji-specific oligonucleotide spans the region of sequence (illustrated in Fig. 3) which includes the additional leucine codon and the Thr→Asn substitution (position 58). (Thus, the Raji-specific oligonucleotide contains 5 out of 17 bases different from the normal c-*myc* sequence, including a 3-base insertion.) With these oligonucleotides as labelled probes for Raji or JI [t(2;8)] mRNA, we detected hybridization of c-*myc* mRNA in both mRNA populations using the common oligonucleotide (Fig. 5*b*) but only c-*myc* mRNA in Raji using the Raji-specific primer, thereby confirming that the latter probe only detects the Raji translocated c-*myc* mRNA in these hybridization conditions. For a quantitative estimation of the contribution of normal or translocated c-*myc* mRNA in Raji cells, the filters were rehybridized with a c-*myc* cDNA clone to allow analysis of the two sets of data by

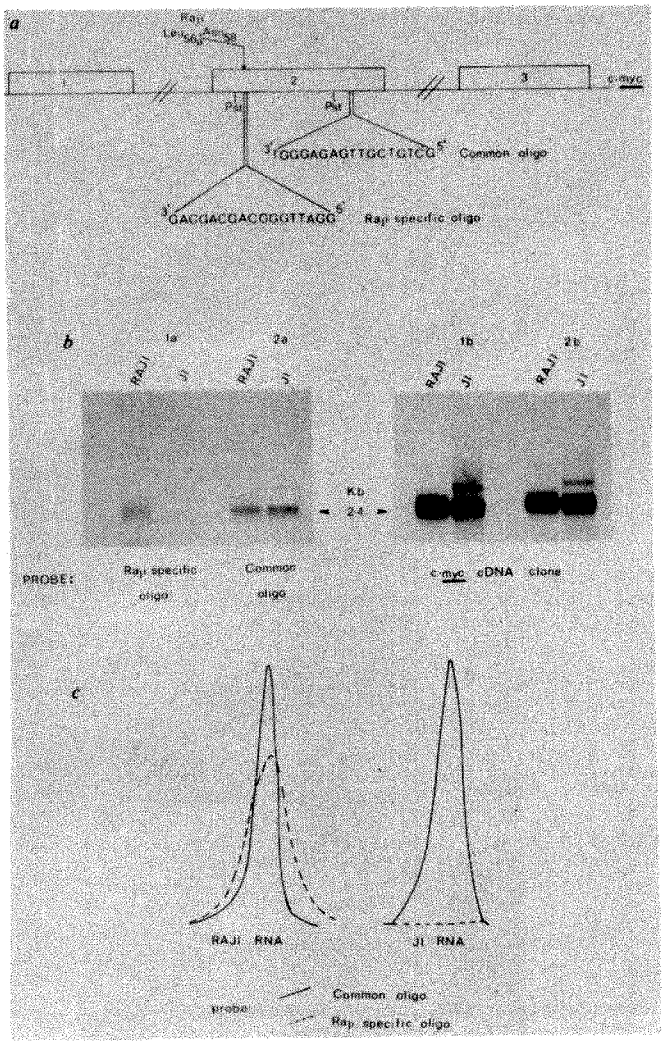


Fig. 5 Northern filter hybridization with c-*myc* oligonucleotide probes. *a*, Diagram of the location and sequence of the common c-*myc* complementary oligonucleotide (the same as used in Fig. 3) and the Raji translocated c-*myc* specific oligonucleotide. *b*, Autoradiographs of filter hybridizations using the various probes indicated. Two aliquots of 50 μg Raji and two of 10 μg JI poly(A)-containing RNA were glyoxylated[41] and run in parallel on a 1.4% agarose gel, followed by electrophoresis and transfer to cellulose nitrate[41] to yield two identical filters, each containing Raji and JI RNA. The filters were hybridized with ^{32}P-end-labelled oligonucleotides at 37 °C for 18 h in 2×SSC containing 0.2% PVP, BSA, Ficol[36], 0.1% SDS and 50 μg ml^{-1} sonicated, denatured salmon sperm DNA. After hybridization the filters were washed at 37 °C with 2×SSC, 0.1% SDS (six times for 10 min each), followed by autoradiography at −70 °C with prefogged film[37]. When the autoradiograph had been obtained, both filters were rehybridized with a cloned c-*myc* probe, pUC cDIA[18]. The relative quantities of RNA in the autoradiographs were assessed by densitometry. *c*, Densitometer tracings from Raji and JI RNA hybridized with the two c-*myc* oligonucleotides.

densitometry; the densitometer tracings of the oligonucleotide hybridizations for JI and Raji mRNA are given in Fig. 5*c*. Taken at face value, the proportion of signal in c-*myc* mRNA from the translocated gene in Raji is about 80%. However, analysis of the intensities of the cloned c-*myc* hybridization suggested lower RNA levels in the Raji lane previously probed with specific oligonucleotide. Normalizing the data shows ~35% untranslocated and 65% translocated c-*myc* mRNA in Raji cells. Therefore, we conclude that both c-*myc* genes are transcribed in Raji cells, unlike other examples of Burkitt's lymphoma, and this unusual transcription of the normal allele correlates with the

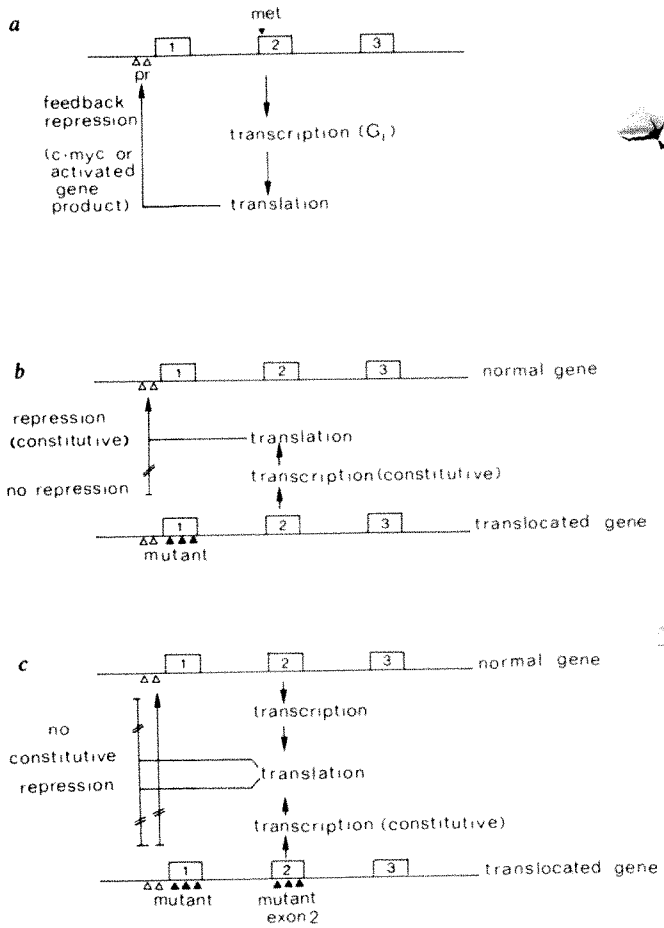


Fig. 6 Diagrammatic scheme of c-*myc* transcriptional control in normal cells (*a*) and Burkitt's lymphoma cell lines (*b*, LY67, Daudi; *c*, Raji). Note that the feedback repressor loop need not necessarily be via the c-*myc* protein itself but could occur via a gene activated by the c-*myc* protein.

presence of the codon replacements (that is, with protein alterations) in the translocated gene.

Immunological implications

In view of the data presented here, it now seems that the translocated c-*myc* very frequently suffers damage as a result of the chromosomal breakage. This is most dramatically demonstrated by studies of the gene in mouse myelomas where there is generally truncation of exon 1[1–4]; in Burkitt's lymphoma, where there is rarely loss of exon 1, there seems to be mutational damage. The difference between mouse and man in this respect is intriguing and may reflect the different rates of somatic mutation, which normally operate on the immunoglobulin variable-region genes, in these two organisms. Comparative hybridization studies of the immunoglobulin genes of mouse and man suggest that there may be as many as 10-fold more *V* genes in the former[27,28]; it was deduced from this that the comparable *V* gene variability was created by an additional mutational load imposed on the *V* genes which rearrange to give active genes in man[27,29]. The results concerning the translocation of c-*myc* support this notion and may explain why the loss of exon 1 is the more common activating event in mouse translocations. Conversely, in man mutational damage within the c-*myc* gene is the most common result, possibly because of the relatively high mutation rate in the B-cell population.

The results on the distribution of mutations and location of the BL translocated c-*myc* gene also have some implications for the mechanism of somatic mutation. Many of the translocations in man involve immunoglobulin C_H genes[11] and the breakage

occurs upstream of the c-*myc* gene (ref. 12 and see Fig. 1). Interestingly, the variant Burkitt's lymphomas break downstream of the c-*myc* gene and involve the proximity of the C_λ gene in t(8; 22)[13,15,16] or apparently the C_κ gene in t(2; 8)[30], although the break has been reported to be near a V_H gene in one case[14]. These results suggest that the common requirement for somatic mutation may be the constant-region gene, either by enzymatic recognition of this sequence as suggested previously[22] or perhaps by the particular chromatin configuration associated with the constant-region gene in these cells. In addition, it seems that the presence of an immunoglobulin constant-region gene near the translocation point is needed for the effect of the translocation to be exerted in the c-*myc* gene.

Model of c-*myc* gene activation

The data presented here show that there are substantial differences between translocated c-*myc* genes in Burkitt's lymphoma compared with normal sequences. These differences presumably arise from somatically introduced mutations associated with the DNA joining during chromosomal translocation and we would argue that the relatively restricted distribution of changes exists because such mutations provide a selective advantage (that is, growth advantage) on the affected cell. Although coding region changes do occur in Raji and LY67 (both within exon 2), as a general rule these are probably not important for oncogenesis (although they may have been in the case of Raji). More significant may be the fact that in the case of Raji, LY67 and JI, the sequence of exon 1 differs from the normal exon 1 sequence. As no coding function can be assigned to exon 1 of c-*myc*, it is possible that this segment, which is well conserved between mice and man[7,12], has a role in the control of transcription of c-*myc*[20]. Our data on the alteration of the sequence of exon 1 support this idea by providing one explanation for the activation of the c-*myc* gene in at least some Burkitt's lymphoma lines even when the chromosomal breakpoint is at some distance from c-*myc*. Similar results have recently been obtained for other Burkitt's lymphoma translocated c-*myc* genes and a similar conclusion was reached[31].

Furthermore, the results support a model in which transcription from the c-*myc* gene can be repressed by the binding of a protein to exon 1 (ref. 21), while explaining the maintenance of exon 1 with the translocated c-*myc* gene, without the requirement for elevated c-*myc* mRNA levels. Figure 6 shows this model, which incorporates our data as well as the observation that the normal c-*myc* allele is usually inactive in cells carrying translocations[12,23]. Figure 6*a* illustrates the normal transcriptional activity of the c-*myc* gene. In the resting cell, c-*myc* gene transcription is minimal but in response to stimulation (for example, mitogen[31]) transcription is initiated from promoter sites upstream of exon 1, followed by protein translation starting in exon 2. The c-*myc* protein product is then envisaged to function by activating a further set of genes (for example, those involved in DNA synthesis) which facilitate cell division. (This transcriptional role for the c-*myc* gene product seems a reasonable supposition as the protein seems to be related to the adenovirus gene E1A[32] which itself displays transcriptional control properties[33].) At the same time, repression of c-*myc* gene activity begins by protein binding (either by direct binding of the c-*myc* protein itself or by a gene product activated by the c-*myc* protein) eventually resulting in the c-*myc* gene becoming quiescent again (in a cyclic fashion). In Burkitt's lymphoma (and mouse myeloma) translocation of the c-*myc* gene seems to subtly disrupt this cyclic transcriptional control. We must also consider the possibility that disruption of control of translocated c-*myc* genes occurs via immunoglobulin locus-activating elements which exert their effects on either exon 1 or some unknown regions upstream of the c-*myc* gene. As indicated in Fig. 6*b*, Burkitt's lymphoma cells (like LY67 and Daudi described here) exhibit constitutive transcription and translation of the translocated c-*myc* gene while at the same time constitutively repressing the normal c-*myc* allele (which, of course, has a normal repressor binding facility). The cell is therefore incapable of returning to the quiescent state of c-*myc* gene activity. The case of the Burkitt's lymphoma cell line Raji is an exception: here the normal allele is not repressed, which provides direct support for the theory that the c-*myc* protein is involved in transcriptional control as we know that the Raji translocated c-*myc* gene is abnormal[22]. Therefore, we envisage that these cells express both c-*myc* alleles (Fig. 6*c*) because mutations in exon 2 of the translocated gene product inactivate or significantly reduce its repressive function. The models predicted here and elsewhere[21,31] are amenable to direct experimental testing, which is now being done using DNA transfer studies of manipulated c-*myc* genes in various types of lymphoid and non-lymphoid cell.

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Observational lower mass limit for black hole formation derived from massive X-ray binaries

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The high radial velocity amplitude (235 km s^{-1}) and 1.70-day orbital period of the B3V optical counterpart of the X-ray source LMC X-3 set a lower limit of 6–10 $M_\odot$ for the mass of the X-ray source, strongly suggesting that it is a black hole[1,2]. An observational upper limit to the mass of this compact star can be derived which, in combination with considerations about the evolutionary history of the system, allows us to derive an upper limit to the (zero age) mass of the progenitor of the compact star in this system: $\lesssim 80\pm10\ M_\odot$. From the orbital parameters of pulsating massive X-ray binaries we find that stars with masses up to $\geq 40\ (\pm5)\ M_\odot$ (and, if supernova (SN) mass ejection is symmetric, up to 60 (±7) $M_\odot$) terminate life as neutron stars. Thus, if LMC X-3 is a black hole, the lower mass limit for terminating life as a black hole is in the range between 40 (60 $M_\odot$) and 80 $M_\odot$. Together with the initial mass function of massive stars this yields a black hole formation rate in the galaxy about two orders of magnitude smaller than the neutron-star formation rate. The direct progenitors of the black holes (just before the supernova) were massive Wolf–Rayet (WR) stars.

From the optical mass function of LMC X-3 (2.3 $M_\odot$), the absence of X-ray eclipses, in combination with the observed spectral type and absolute visual magnitude of the B-type companion, Paczynski[2] derived a lower limit to the mass of the B-star of 9.0 ($\pm6.0\ M_\odot$), that is $\geq 3.0\ M_\odot$. By comparing the observed absolute magnitude of this star with theoretical evolutionary tracks, he obtained an upper mass limit of 6.6 (±2.0) $M_\odot$, that is $\leq 8.6\ M_\odot$. Thus, the allowed mass-range for the B-star is 3.0–8.6 $M_\odot$. A lower limit to the mass M_H of the compact star is set by the absence of X-ray eclipses[2] which yields $M_H \geq 6.1$ and $\geq 10.0\ M_\odot$, for $M_B = 3.0\ M_\odot$ and 8.6 $M_\odot$, respectively. Upper limits to M_H are set by the lower limit to the orbital inclination (i) derived from: (1) the observed rotational broadening of the spectral lines of the B-star, which yields $i \geq 50°$ (ref. 1), and (2) the amplitude of the photometric variability of this star which, yields $i \geq 55°$ (ref. 3). Adopting $i \geq 50°$ as a safe lower limit we obtain $M_H \leq 9.0\ M_\odot$ for $M_B = 3.0\ M_\odot$ and $M_H \leq 13.6\ M_\odot$ for $M_B = 8.6\ M_\odot$. (For the shape of the allowed domain of M_B, M_H: see ref. 1.) The above derived largest and smallest M_B, M_H combinations are listed in the first two columns of Table 1. For a certain (M_B, M_H) combination one can derive an upper limit for the mass of the progenitor of the black hole just before its core collapse, as follows. Denoting the mass of the collapsed star before the SN as M_1 and assuming an initially circular orbit and instantaneous spherically symmetric ejection (that is, in a time interval shorter than the orbital period) of the SN shell (of mass $\Delta M = M_1 - M_H$) the post-SN orbital period, semi-major axis and orbital eccentricity are given by[4]

$$P_f = (\mu_f/(2\mu_f - 1)^{3/2})P_0; \qquad a_f = a_0\mu_f/(2\mu_f - 1);$$
$$e_f = (1-\mu_f)/\mu_f \tag{1}$$

where subscripts zero and f denote the situation just before and after the SN, respectively, and $\mu_f = (M_H + M_B)/(M_1 + M_B)$ (we neglect the effects of the impact of a small fraction of the SN shell onto the B-star, as these as well as those of asymmetries are expected to be small[5,6].

As the present orbital period is only 1.70 days, tidal forces in the system are strong, and are expected to have rapidly circularized the orbit after the SN[7]. After tidal circularization the semi-major axis has decreased by a factor $(1 - e_f^2)$ (we neglect the spin angular momentum of the B-star which is several orders of magnitude smaller than the orbital angular momentum). Using this, and applying Kepler's third law one finds that the orbital period P' and separation a' after tidal circularization are related to the pre-SN values by

$$P' = P_0/\mu_f^2,\ a' = a_0/\mu_f \tag{2}$$

Because $P' = 1.70$ days, one thus can calculate P_0 for any assumed combination of M_B, M_1 and M_H. A strong constraint to the pre-SN mass M_1 of the compact star is set by the fact that, before the SN, the radius of the B-type star cannot have exceeded that of its Roche lobe, R_R, though must, on the other hand, at least have been as large as its zero age main sequence (ZAMS) value R_0.

Using the expression for the Roche lobe given by Eggleton[8], the condition that before the explosion R_0 was $\leq R_R$, in combination with the above equations yields the following condition for μ_f

$$\mu_f f^{2/3}/\{0.6f^{2/3} + \ln(1+f^{1/3})\} \geq \frac{R_0}{R_B}\left\{\frac{q^{2/3}}{0.6q^{2/3} + \ln(1+q^{1/3})}\right\} \tag{3}$$

where $f = \mu_f/(1/q - \mu_f + 1)$, $q = M_B/M_H$ is the present mass ratio of the system, and R_B is the present radius of the B-star. R_B can be simply calculated, for the given M_B, M_B combination, from the fact that the B-star fills its Roche lobe in the present orbit.

For a given (M_B, M_H) combination, q and all quantities on the right-hand side are known, such that the lower limit on μ_f can be solved. The resulting limiting values of μ_f, M_1, e and P_0 for the lower and upper limits of the range in (M_B, M_H) values are given in Table 1. For R_0 we used the values at an age of 3×10^6 yr—the approximate age at which the companion exploded—calculated by Van der Linden[10] for $X = 0.70$, $Z = 0.01$ (the approximate metal abundance of the LMC). These are

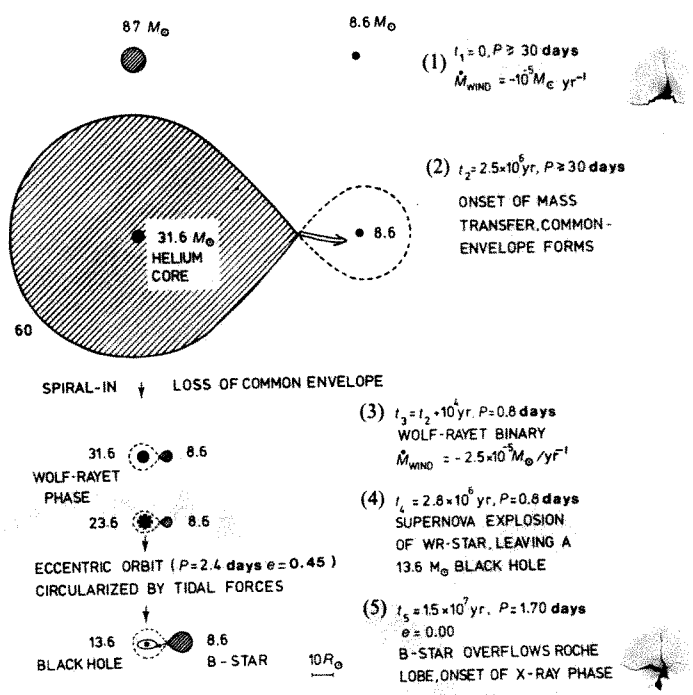


Fig. 1 Maximum-progenitor-mass evolutionary model for the origin of the LMC X-3 system, corresponding to the maximum possible masses of the compact star and its companion as allowed by the observations. Between stages (1) and (2) and between (3) and (4) the massive star undergoes stellar wind mass loss. Scale of (3), (4) and (5) is twice that of (1) and (2).

Table 1 Upper limits to the immediate pre-supernova mass M_1 of LMC X-3, and corresponding limits on μ_f, P_0, a_0, e, R_1 derived for the minimum and maximum possible values of the component masses M_B and M_H; also shown are the range of helium-core masses and initial (zero-age) primary masses M_i corresponding to the M_1 values

	M_B ($M_\odot$)	M_H ($M_\odot$)	R_0 ($R_\odot$)	R_R ($R_\odot$)	μ_f $\geqslant$	M_1 ($M_\odot$) $\leqslant$	P_0 (day) $\geqslant$	a_0 ($R_\odot$) $\geqslant$	e $\leqslant$	R_1 ($R_\odot$)	M_{He} ($M_\odot$) $\leqslant$	M_i ($M_\odot$) $\leqslant$
Minimum	3.0	6.1	1.7	4.02	0.53	14.3	0.47	6.6	0.9	3.4–5.5	14.3–19.0	44.0–58.7
Maximum	8.6	13.6	3.47	5.75	0.69	23.6	0.81	11.7	0.45	5.5–7.2	23.6–31.5	69.4–86.9

Table 2 Lowest-progenitor mass evolutionary history for the 4U1223−62 system, taking minimum rates of mass loss by stellar wind and during the exchange stage into account, together with the evolutionary model for the 4U1223−62 system if the supernova mass ejection was spherically symmetric (to produce $e = 0.47$)

Lowest-progenitor mass evolutionary model for 4U1223−62 (asymmetric supernova)*			Evolutionary stage	Evolutionary model for 4U1223−62 with symmetric supernova†		
Approximate age (10^6 yr)	$M_1(M_\odot)$	$M_2(M_\odot)$		Approximate age (10^6 yr)	$M_1(M_\odot)$	$M_2(M_\odot)$
$t = 0$	42	38	(1) Zero age	0	66	34
3.9	34	31.2	(2) Onset of mass transfer	3.3	49	31
$3.9 + 10^4$ yr	15	44	(3) End of mass transfer	$3.3 + 10^4$ yr	28.5	45
4.35	10	43	(4) Supernova	3.59	22.7	44
4.35	1.4	43	(5) Post-supernova	3.59	1.4	44
5.2	1.4	40	(6) Present (X-ray phase)	5.0	1.4	40

* At the onset of the (case B) mass transfer the component masses have decreased to 34 $M_\odot$ + 31.2 $M_\odot$ due to stellar wind mass loss. Some 6 $M_\odot$ is assumed to be lost during the exchange. During the $\sim 4.5 \times 10^5$ yr of helium burning and subsequent stages (between stages 3 and 4) the 15 $M_\odot$ WR star is assumed to lose 5 $M_\odot$, reducing its mass to 10 $M_\odot$ at the time of the supernova. The 44 $M_\odot$ companion needs about 1.3×10^6 yr after the mass exchange to terminate its hydrogen burning during which it loses $\sim 4 M_\odot$ by wind. This leads to the present system, consisting of a $\sim 1.4\ M_\odot$ neutron star and a 40 $M_\odot$ blue supergiant. In order to explain the observed orbital eccentricity ($e = 0.47$) the supernova mass ejection must have been very asymmetric in this model.

† Stellar wind mass loss and mass loss during the exchange was taken into account in the same way as in the other model (see text). If no mass loss occurs during the exchange, the zero age masses of the progenitor and its companion become 56 $M_\odot$ and 33 $M_\odot$, respectively.

listed in the third column. For any other (M_B, M_H) combination in the allowed range, the M_1 values are between those listed in Table 1. Notice from Table 1 that the lowest allowed value for μ_f is 0.53, which is consistent with the fact that disruption can have occurred only if $\mu_f \leqslant 0.50$ (ref. 9). The M_1 values in Table 1 are upper limits as we neglected the effects of impact, which would have further slightly enlarged e and P_f and thus would have lowered the maximum allowed M_1 values[5].

The second step is to relate these M_1 values just before the SN, to the initial (zero age) mass of the progenitor. The very short orbital period and the large mass of the pre-SN star indicate that the pre-SN star must already have been compact (see the pre-SN Roche-lobe radii R_1 of the progenitor of the compact star in Table 1): a very evolved star of $\sim 20 M_\odot$ with a radius of only $\sim 5\ R_\odot$ cannot have had any hydrogen envelope left, because such an envelope around a heavy-element core immediately expands to a large radius. Hence, the pre-SN star must have consisted of helium and heavier elements. A helium core of about 20 $M_\odot$ requires an initial stellar mass of at least about 50 $M_\odot$. Hence, the mass ratio of the initial zero age system was of the order of 0.1. In systems with initial mass ratios $\leqslant$0.3–0.4 the onset of mass transfer from the more massive (and thus more evolved) component to the less massive one leads to the formation of a common envelope in which the core of the massive star and the companion spiral in towards each other[11–13]. Figure 1 (1)–(3) depicts this type of evolution schematically. The common envelope is lost on a short time scale ($\leqslant 10^4$–10^5 yr) as a consequence of large frictional heating[13,14]. The very short present orbital period of LMC X-3 (and the even shorter pre-SN orbital period) clearly indicate that such spiral-in must have taken place here[2]. (This evolution is the high-mass analogue of the formation mechanism of cataclysmic-variable binaries, see refs 12, 13.) After the spiral-in only the compact core of the massive star consisting of helium and possibly heavier elements, remained, with an orbital period of the order of a day or less. Stripped evolved cores of massive stars are thought to be identified with WR stars[15,16]. Such stars have strong stellar winds, with mass loss rates $\dot{M} \sim (1\text{–}5) \times 10^{-5}\ M_\odot\ \mathrm{yr}^{-1}$ (refs 17, 18). During its lifetime of $\sim 3 \times 10^5$ yr (for $M_{He} \approx 25\ M_\odot$) such a star may lose some 6 $M_\odot$, so that the products of core-helium burning may become exposed at the surface, turning the star into a so-called WC star[19,20]. The progenitor of the black hole in LMC X-3 just before the SN probably was such a WC star. The presence of helium in the spectra of WC stars shows that near the end of their nuclear evolution, when their heavy-element cores occupy 75% of their initial mass[21], these stars have not yet been stripped entirely of their helium-rich outer layers. Hence, their mass at the time of the SN is $\geqslant$0.75 times the initial helium-core mass. This allows one to convert the pre-SN mass limits M_1 in Table 1 into upper limits on the helium-core mass of the progenitor. This yields the maximum M_{He} values in Table 1; the lower limits to M_{He} are equal to M_1.

In turn, these M_{He} values can be converted into initial (zero age) stellar masses, by using the relation between initial stellar mass and helium-core mass, resulting from stellar-evolution calculations in which the effects of stellar wind mass loss during core-hydrogen burning are taken into account. The calculations by Chiosi *et al.*[22] with mass loss parameter $\alpha = 0.83$, closely reproduce the observational estimates[23] of the amount of mass lost by massive stars during the core-hydrogen burning phase, and yield the relation

$$M_{He} = 0.1015\ (M_i)^{1.285} \qquad (4)$$

(for $X = 0.70$; for lower X values, M_{He} increases[24]).

This yields the maximum values for the initial mass M_i given in Table 1, in the range 69.4–86.9 $M_\odot$. Effects of convective overshooting, a smaller initial hydrogen abundance, and so on[19,20,25,26], all result in increasing the fractional mass of the helium core over that given by equation (4), and result in lower initial mass values for the progenitor of LMC X-3. We thus conclude that the upper limit to the mass of this progenitor is about 80 (± 10)$M_\odot$. Figure 1 depicts the above described

evolutionary history of the LMC X-3 system for the maximum possible progenitor mass of ~87 $M_\odot$.

An estimate of the upper mass limit for stars that terminate their evolution as neutron stars can be derived from the presence of an X-ray pulsar in the most massive known X-ray binary system 4U1223−62. The X-ray mass function of this 41.4-day orbital period system is 31 $M_\odot$ (refs 27–29). Together with the absence of X-ray eclipses—which sets an upper limit to the orbital inclination—this leads to a mass $\geq 40\ M_\odot$ for the B1.5 Ia supergiant companion[27,30]. To derive a lower limit for the mass of the progenitor of the neutron star in this system we have to consider evolution with mass exchange. To include the effects of stellar wind mass loss, we have again adopted the wind mass loss rates for hydrogen-burning O and B stars from ref. 22, as fitted by the parameter $\alpha = 0.83$. These rates are lower than given by most authors[19,20] and are therefore suitable for deriving a lower mass limit for the progenitor. As to the mass loss from the system during the exchange: a comparison between the distributions of the mass ratios of the WR-binaries and their progenitors, the O-type spectroscopic binaries, shows that at least 30% of the envelope of the primary star is lost during the exchange[32,33]. Using the condition that we wish to avoid, tandem evolution (see ref. 37), we find that the lowest-mass realistic progenitor system of 4U1223−62 has components of 42 $M_\odot$ and 38 $M_\odot$. Table 2 lists the component masses at various evolutionary stages. As we have adopted the lowest possible mass loss rates compatible with the observations (both by wind and during the exchange) it seems that 42 $M_\odot$ is about the lowest possible progenitor mass for the neutron star. If no mass loss during the exchange were adopted (but wind mass loss still is included) the smallest-mass progenitor system would be 37 + 33 $M_\odot$. Taking uncertainties in, for example, wind mass loss rates and overshooting, into account—the resulting lower limit to the mass of the progenitor of the pulsar 4U1223−62 is $40 \pm 5\ M_\odot$.

With the progenitor system of Table 2 it is impossible to explain the observed orbital eccentricity $e = 0.47$ of the 4U1223−62 system unless the supernova mass ejection was highly asymmetric. For spherically symmetric ejection the eccentricity expected from equation (1) is only 0.19. (Due to the extensive mass exchange the pre-SN orbit is expected to have been circular.) If the SN mass ejection is assumed to have been spherically symmetric, the observed eccentricity $e = 0.47$ together with equation (1) yields a mass of 22.7 $M_\odot$ for the progenitor of the neutron star at the time of the explosion. The evolutionary history of the system for this case is listed in Table 2 and the corresponding zero age mass of the progenitor seems to be about 60 (±7) $M_\odot$.

Combining the results of Tables 1 and 2 we conclude that the lower (zero age) mass limit for stars which terminate their evolution as a black hole is in the range between 40(±5) $M_\odot$ and 80 (±10) $M_\odot$. If SN mass ejection is spherically symmetric this limit is between 60 (±7) and 80 (±10) $M_\odot$. We expect these limits also to be good approximations for single stars, as single stars with masses $\geq 40\ M_\odot$ have very large rates of wind mass loss and are expected to turn into WR stars and evolve in a way similar to that of binary components[15,19,20]. Knowledge of this limit is of great importance for estimating the bulk yield of nucleosynthesis by massive stars[21].

According to Garmany *et al.*[34] the number of O and WR stars originating from stars more massive than 40 $M_\odot$, 60 $M_\odot$ and 80 $M_\odot$ within 2.5 kpc from the Sun is between 100 and 132, between 25 and 55, and between 8 and 10, respectively.

Assuming mean lifetimes of 2.5×10^6 yr, 3×10^6 yr and 3.5×10^6 yr for stars more massive than 80 $M_\odot$, 60 $M_\odot$, and 40 $M_\odot$ respectively, this yields a black hole formation rate in the solar neighbourhood in the galactic plane of $(0.2, 0.9 \text{ and } 2.0) \times 10^{-6}$ kpc^{-2} yr^{-1}, respectively. This is about two orders of magnitude smaller than the galactic SN rate[35] and the pulsar birth rate[36].

Finally, because about half of all black holes are expected to be formed in binaries, a better knowledge of the number of black hole X-ray binaries in the galaxy, in combination with the initial mass function, will allow an independent estimate of the lower mass limit for black hole formation.

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Discovery of M87-like ejection in F-71

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The M87 jet[1,2] is one of the most obvious manifestations of violent activity inside the compact nuclei of galaxies whilst also being one of the most spectacular phenomena of extragalactic astronomy. Although many radio jets are known, very few optical jets have been detected and virtually none is comparable to the prototype jet in M87. Recent charge-coupled device (CCD) images of the southern object F-71 show that this elliptical galaxy ($z = 0.057$) exhibits a brilliant knot ~14 arc s away from the nucleus and is physically connected to it. Optically, this knot resembles in many respects the bright knots A and B in the M87 jet: its spectrum is featureless and very blue with a slope ($\alpha = 1.7 \pm 0.1$) equal to that of the M87 knots. Its relative luminosity with respect to, and its separation from, the nucleus are both greater than in the M87 galaxy–jet system. We suggest here that the most likely interpretation of the F-71 knot is that it is ejected from the parent galaxy in the same way as the knots in the M87 jet. We propose that further observations of the F-71 system might provide important clues to our understanding of the ejection activity from the nuclei of galaxies.

The object F-71 ($\alpha_{1950} = 20$ h 14.8 min, $\delta_{1950} = -57° 43'$) from Fairall's list[3] of compact galaxies appears as an almost circular galaxy of approximate magnitude 16 mag on the European

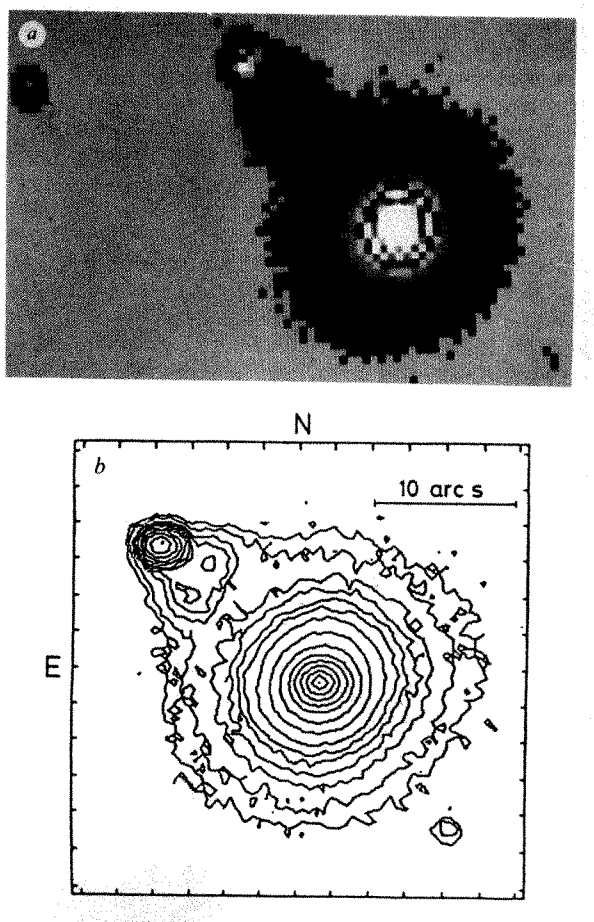


Fig. 1 *a*, CCD image in the *r*-band of F-71 showing the connection between the nucleus of the elliptical galaxy and its nonthermal knot. *b*, Contour plot of the CCD image in the *r*-band of F-71.

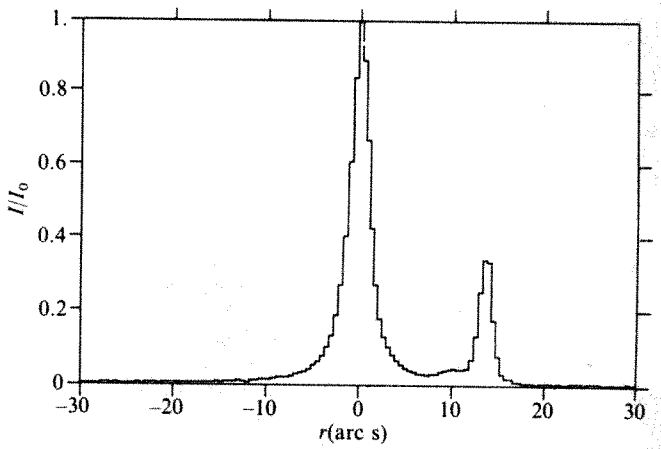


Fig. 2 Tracing through the nucleus of F-71 and the north-east knot in the *r*-band.

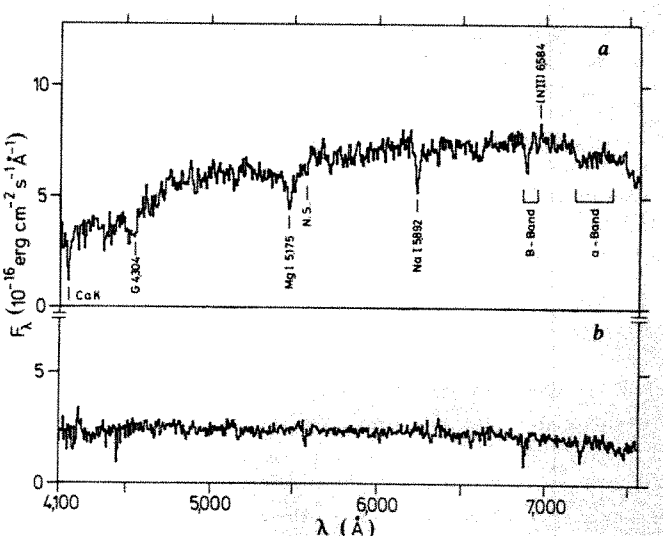


Fig. 3 The spectra of the nucleus of F-71 (*a*) and of the north-east knot (*b*).

Southern Observatory (ESO) Quick Blue Survey plate. Towards the north-east of the object a starlike companion is visible approximately 10 arc s away which has not been described before. CCD images of F-71 have been obtained on 7 and 8 May 1983 with the 1.5-m Danish telescope at La Silla (exposure time 45 min, seeing 1–2.5 arc s, 1 pixel ≙ 0.471 arc s) through various filters (*B*, *V*, *r*, *z*). All these images reveal a physical connection between the north-east knot and the parent galaxy.

As an example, we display our observational results for the *r*-band: the CCD direct image (Fig. 1*a*) shows the connection between the knot and the nucleus. The isophote representation (Fig. 1*b*) and the intensity tracing through the nucleus and the knot (Fig. 2) reveal the light distribution in more detail. The brightness distribution of the parent galaxy closely follows a de Vaucouleurs law, and is classified as an elliptical galaxy of type E0–E1. Its minor axis points in the direction of the optical north-east knot, which is located 13.75″ ± 0.3″ away from the galaxy centre in position angle (PA) 49.5° ± 0.5°. A possible weak 'counter-jet' is indicated in the direction 221.5° ± 0.5° and 12.65″ ± 0.3″ away from the galaxy centre, and shows up particularly well in the red bands *r* and *z*. The north-east knot appears slightly extended on the far side compared with stellar images on the same CCD-frames. On the near side, the optical isophotes in all filter bands show a backward extension of the compact north-east knot nearly symmetrically with respect to the line joining the nucleus and the knot (see Fig. 1 for the *r*-band). This feature is reminiscent of the "waste energy baskets" described by Longair *et al.*[4] in connection with the radio hotspots of extended radio galaxies, in particular of Cyg A. The detection of this tailed structure in all filter bands supports a physical connection between the blue knot and the galaxy as opposed to the possibility of a chance superposition of F-71 and a background object. From the tracings through the galaxy and the north-east knot we derive relative peak intensities of the knot with respect to the galaxy centre for the *z*, *r*, *V* and *B* bands, increasing towards the blue as follows: 0.31, 0.36, 0.37 and 0.53.

Optical spectra of both the galaxy and the north-east knot of F-71 were obtained on 2 September 1983 at the ESO 3.6-m telescope equipped with the Boller and Chivens spectrograph and the IDS (entrance aperture 4″ × 4″, dispersion 171 Å mm^{-1}, wavelength range 4,100–7,300 Å, exposure time 20 min each). The galaxy spectrum (Fig. 3*a*) shows absorption lines typical of elliptical galaxies. Fairall suspected, on the basis of his low-dispersion slitless spectrum of F-71, the presence of Balmer emission features. From our spectrum we cannot confirm this conjecture. Hα is coincident with the atmospheric B-band. There is marginal evidence for the presence of weak [N II] 6,584 emission. From the absorption lines we derived a redshift $z = 0.0573$ which puts F-71 at a distance 229 Mpc ($H_0 = 75$ km s^{-1} Mpc^{-1}). The visual apparent and absolute magnitudes derived from the flux at 5,500 Å are $m_V = 16.8$ mag, and $M_V = -20.0$ mag. With a scale 1.1 kpc arc s^{-1} the north-east knot is at a projected distance 15.3 kpc from the galaxy centre.

The optical spectrum of the knot appears to be featureless and flat (see Fig. 3*b*) but does not entirely rule out the possibility that the spikes in the short wavelength end of the spectrum at λ 4,216 Å and λ 4,453 Å are real and are [O II] λ 3,727 and

CaK λ 3,934 of a BL Lac object at $z = 0.13$. However, in view of the morphological evidence described above and shown in Figs 1 and 2, this interpretation does not seem to be tenable. The mean spectral index of the optical continuum of the knot is $\alpha = 1.7 \pm 0.1$ ($F_\nu \propto \nu^{-\alpha}$). The tracings through the nucleus and the knot of F-71 show that in all the colour bands the relative brightness and concentration of light between the jet and nucleus is very similar to those in M87 and its jet[1]. This may be taken as further evidence that both phenomena have the same physical origin.

F-71 and its north-east knot resemble in many respects the situation of the giant elliptical galaxy M87 with its spectacular nonthermal jet. Like M87, F-71 is an elliptical galaxy of the type E0–E1. In M87 the one-sided jet is made up of several nonthermal knots, while in F-71 a single strong knot is visible to one side and a weak feature to the opposite side. The flat and apparently lineless spectrum of the north-east knot corresponds to the nonthermal spectrum of the knots A and B of M87 displayed by Sulentic *et al.*[2]. Intriguingly, the spectral slope $\alpha = 1.7 \pm 0.1$ of the F-71 knot continuum matches exactly that of the knots A and B in the M87 jet, $\alpha = 1.7 \pm 0.2$ (ref. 5). Intrinsically, the scale of the F-71 phenomenon is an order of magnitude larger than in the M87 prototype. In F-71 the north-east knot is much more clearly separated from the main body of the galaxy than the jet in M87, and its luminosity, relative to the galaxy, is much stronger.

The optical data on F-71 described here characterize this source as a galaxy with similar ejection activity as M87, thus warranting further extensive studies, in particular in the radio region.

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Conjugate ionospheric flows on Uranus

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Consideration of the pointing direction and rotation period of the spin of Uranus suggests that a novel kind of day-to-night ionospheric flow system may exist there. For equatorial distances $r < r_c$ ($\approx 2.74 r_p$), the conjugate ionospheric flow from the dayside hemisphere to the nightside hemisphere could become supersonic as it crosses the equator and a shock forms at the termination point; for $r > r_c$, smooth transition at the contact point between the ionospheric flow from the dayside hemisphere and the nightside atmosphere is possible. The rotational effect of Uranus also limits the inner extension of the thermal plasma disk generated from energetic particle sputtering of the ring-particles and satellites. The combined effect of the dynamic transport of the ionospheric plasma and the injection of ring plasma into the upper atmosphere could provide interesting results concerning the ionosphere of Uranus, which is to be explored by the Voyager 2 spacecraft in 1986.

As a result of the slow rotation of Venus, its nightside atmosphere is not subjected to a photoionization effect; however, spaceprobes to Venus have found that it has a very dynamic and sizeable nightside ionosphere[1,2]. Its maintenance has been suggested to result from impact ionization by energetic electrons (100 eV–1 keV) accelerated in the magnetic tail[3,4], or from transport of ionospheric plasma from the dayside to the nightside[1,5]. The ionospheric transport process of Venus, driven by solar heating and solar wind interaction directly with the dayside atmosphere, is unique among the planetary ionospheres. Note that a similar day-to-night ionospheric flow pattern could also occur at Uranus because although Uranus has a rapid spin period (16.31 ± 0.27 h; see ref. 6) its rotational axis is pointing towards the Sun. This means that while one hemisphere is illuminated constantly by sunlight the other is always in darkness. Therefore, only the dayside atmosphere is affected by photoionization—just as for Venus. One major difference between Venus and Uranus, however, is that while Venus has little or no intrinsic magnetic field, Uranus should possess an intrinsic magnetic field of significant strength as recently indicated by the UV observations of enhanced H Lyα emissions at Uranus[7–9]. In fact, Hill *et al.*[10] have postulated that the equatorial surface dipole field should be ≈ 4 G.

The expected hemispherical flows in the closed field-line region are shown in Fig. 1. In the simplest situation, the photoelectron–H^+ flux streaming out of the dayside hemisphere along a flux tube will cross the equator and then be absorbed at the conjugate point.

Rösler[11] has discussed the presence of such conjugate-point ionospheric flows at the terrestrial ionosphere during solstices at high altitudes. The occurrence of this type of inter-hemispheric flow should be much more widespread at Uranus as a result of the peculiar orientation of its rotational axis at present. Following the standard treatment in solar wind theory, the hydrodynamic equations describing the ionospheric flows (assumed to be isothermal) can be combined into[12]:

$$\frac{1}{2}(M^2 - M_0^2) - \ln\left(\frac{M}{M_0}\right) - \ln\left(\frac{A}{A_0}\right) + \frac{\phi - \phi_0}{c_0^2} = 0 \qquad (1)$$

where $M = u/c$ is the ratio of the flow speed to the sound speed given as $c^2 = 2kT/m$ (kT is the plasma temperature, and m the proton mass), and A is the cross-sectional area of the flux tube. Also we have

$$\phi_g = -\frac{GM}{r} \qquad (2)$$

$$\phi_c = -\frac{\Omega^2 r^2 \cos^2\theta}{2} \qquad (3)$$

where G is the gravitational constant, M the planetary mass ($= 8.7 \times 10^{28}$ g), r the radial distance, θ the latitudinal angle, and Ω the angular rotation frequency ($\Omega = 1.07 \times 10^{-4}$ rad s^{-1}). In

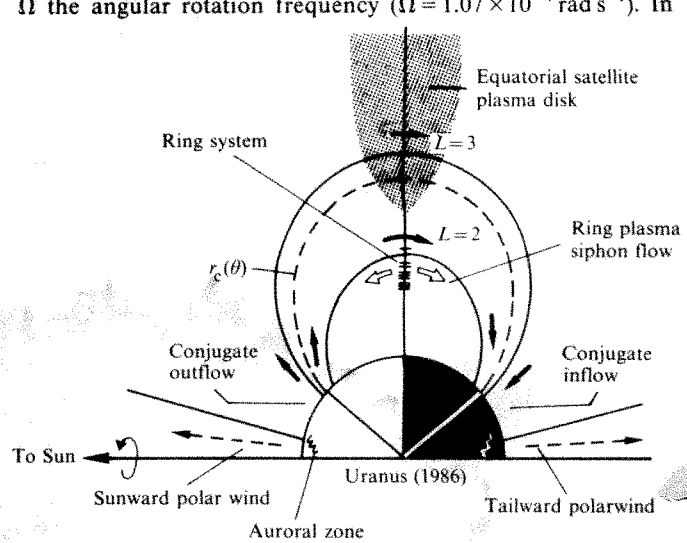


Fig. 1 The conjugate ionospheric flow pattern at Uranus. Such inter-hemispheric flows could coexist with a siphon flow of thermal plasma originates from the equatorial region as a result of energetic charged sputtering, meteoroid bombardment, and photosputtering of the rings and satellites.

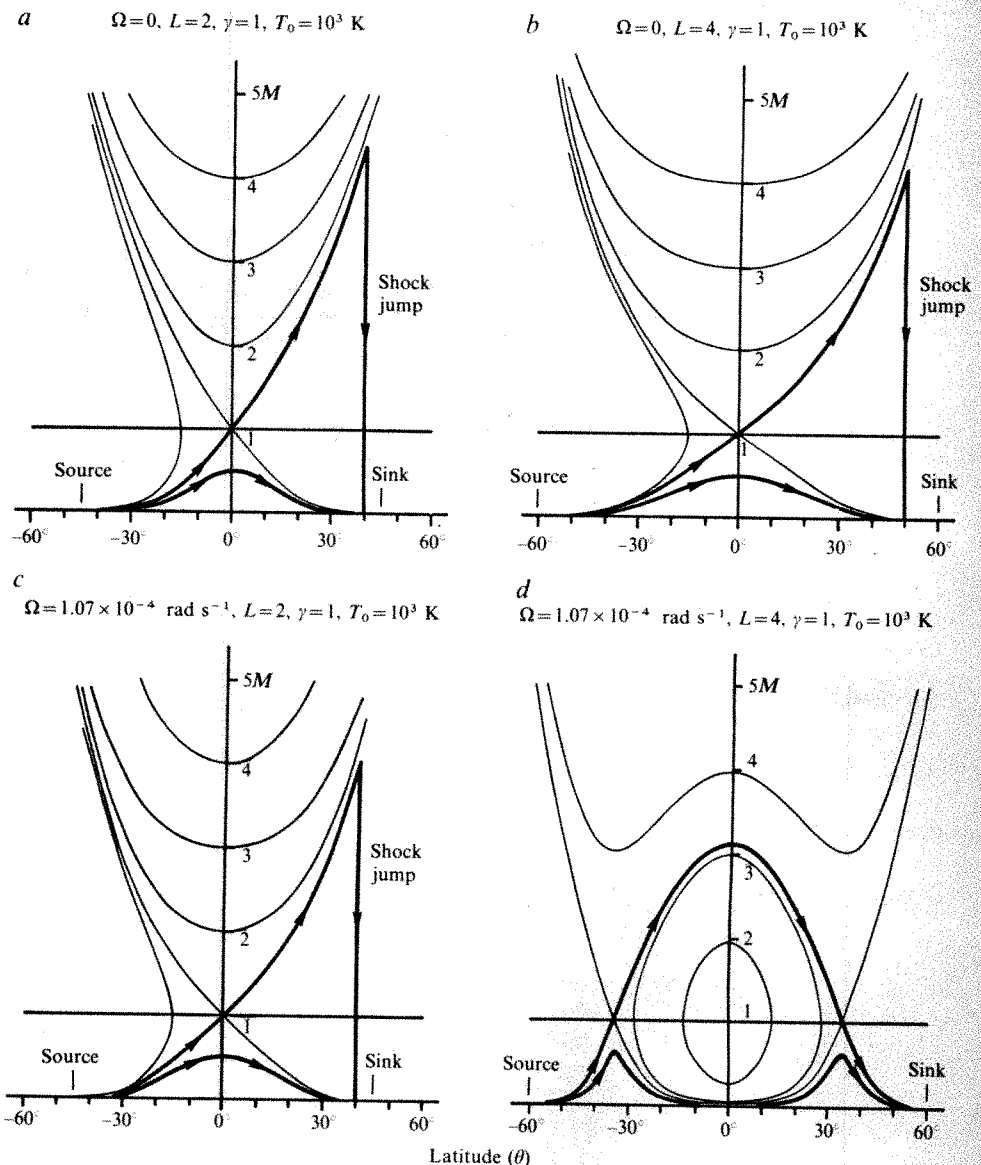


Fig. 2 Examples of isothermal conjugate ionospheric flows at Uranus with the plasma temperature set at $T = 1{,}000$ K. Four cases are considered: *a*, non-rotating case (angular frequency $(\Omega) = 0$) and the field-aligned flow crosses the equator at a radial distance of $2.0r_p$ ($L = 2.0$); *b*, $\Omega = 0$ and $L = 40$; *c*, $\Omega = 1.07 \times 10^{-4}$ rad s^{-1} (rotation period = 16.31 h) and $L = 2.0$; *d*, $\Omega = 1.07 \times 10^{-4}$ rad s^{-1} and $L = 4.0$.

equation (1) $\phi = \phi_g + \phi_c$ and the subscript (0) denotes values corresponding to radial distance r_0 and latitude θ_0 ($r = r_e \cos^2 \theta$ where r_e is the radial distance at the equator).

In the case of a non-rotating planet, several solutions to equation (1) are possible. These include: (1) transition from subsonic flow to supersonic flow before reaching the equator and then going through the reverse transition at the corresponding conjugate point; (2) reaching the sonic point at the equator and then retaining supersonic flow speed all the way to the other hemisphere; (3) retaining subsonic speed at all positions.

All of these solutions could be generated by adopting different sets of pertinent parameters (such as initial flow speed and plasma temperature) for the hydrodynamic flows. A more complete description of them will be presented elsewhere. We have depicted only a subset in Fig. 2 to demonstrate the idea of a conjugate ionospheric flow as well as the potential importance of the planetary rotation in modifying such flows. Figure 2 shows how the ionospheric flow from the dayside hemisphere might go through a sonic point as it crosses the equator or it might remain subsonic all the way to the nightside hemisphere if it starts with a smaller Mach number. In the supersonic flow case, a shock forms at the termination point as the ionospheric plasma slams into the darkside atmosphere. Such flow patterns hold for a wide range of radial distances as shown in Fig. 2*a*, *b*. However, for Uranus, there is an important modification to the flow dynamics at large radial distances; this is because the rapid rotation of the planet means that centrifugal effects begin to have a role in the force balance beyond a certain point. In the simplest possible situation, let us consider first the motion of one single charge particle. In a cold plasma approximation, neglecting the magnetic moment, the balance[13,14] between gravitational and centrifugal accelerations at the equator will define a radial distance (in the case of a perfect dipole field)

$$r_c(\theta) = \left[\frac{2GM \sec^2 \theta}{3\Omega^2}\right]^{1/3} \quad (4)$$

such that the charged particles with zero magnetic moment will be kept to the equator when $r > r_c$ but will be pulled to the topside atmosphere when $r < r_c$. Because r_c $(\theta = 0) = 6.96 \times 10^4$ km $(= 2.74r_p)$ we have $\theta_c = 53°$.

In the case of the ionospheric flow, a similar limit could apply. Figure 2 provides the most extreme examples of the modification effect by the centrifugal force on the conjugate ionospheric flow. Briefly, for flows with sonic point at the equator, those originating from latitudes $\theta < \theta_c$, the 'X-point' configuration near the equator remains unchanged. On the other hand, for those originating from latitudes $\theta > \theta_c$, the corresponding X-point configuration could be substituted by the O-point configuration such that the plasma flows should be subsonic as they reach the other hemisphere (see Fig. 2*c*, *d*). Note that the general nature of an O-type flow in a non-rotating planet remains unchanged if the rotational motion of the planet is introduced. In addition to these many possible variations in the flow patterns, the net results are that the dayside upper ionosphere should be constantly in a state of dynamic expansion instead of diffusive

equilibrium, and that the nightside atmosphere will receive a supply of ionization in this way, and a nightside ionosphere may be maintained accordingly.

In the case of Saturn, it has been postulated that the ionospheric region with magnetic field lines touching the rings should also approach the condition of free streaming—as a result of the absorption effect of the rings[15]. Because of the narrowness of the rings of Uranus, they would be less efficient in depleting the ambient plasma density in the vicinity of the ring system. The ε ring with a width of 50–100 km, however, should be able to intercept the ionospheric plasma and convert the hydrogen ions to neutralized H atoms. The ε ring should also act as an absorbing barrier in keeping the magnetospheric energetic charged particles from diffusing inward further than $2.4r_p$ (uranian radius). Cheng and Lanzerotti[16] have suggested that energetic particle sputtering of the rings could be an important cause of the thermal plasma in the magnetosphere. The associated production of the sputtered neutrals will generate a thin neutral cloud, reflecting the composition of the ring particles. The subsequent ionization of these heavy neutrals (that is, CH_4, H_2O and their fragments) will form a plasma disk at the equator. Force balance relation as considered in previous studies[14,17] indicates that the thermal plasma disk (if it exists) could be essentially limited to radial distances outside r_c. In other words, as shown in Fig. 1, a 'siphon' plasma flow from the equatorial region to the upper atmosphere should be expected in addition to the conjugate ionospheric flow. The dynamic transport of ionization from one hemisphere to another and the simultaneous loading of the atmosphere with hydrocarbons from the rings could have rather interesting effects on the ionospheric system of Uranus.

Finally, questions may be raised about how the presence of a plasma torus might affect the conjugate flow pattern. The most obvious effect is that the angular velocity of the magnetospheric plasma may be reduced to a value smaller than what is expected of rigid co-rotation, and the corresponding centrifugal force may be less important. In addition, the magnetic field configuration might be significantly changed from that of a dipole field.

Two mechanisms could be relevant here. First, as discussed previously[18] for the case of a rapidly-rotating magnetosphere[18,19], if the plasma density is large enough such that at a certain point the kinetic energy of the co-rotation motion exceeds that of the (dipolar) magnetic field energy, namely $\rho V^2/2 \geq B^2/8\pi$, no rigid rotation will be enforced; instead a radial outflow of the magnetospheric plasma stretching the magnetic field outward is expected to occur beyond this critical point. For Uranus, to reach this condition at $L \approx 4$ requires the plasma number density (assumed to be O^+ ions) to exceed $n_c \approx 10^7\ cm^{-3}$ if the equatorial surface field $B_0 \approx 4$ G, and $n_c \approx 4 \times 10^4\ cm^{-3}$ at $L \approx 8$ (the critical values of n_c are a factor of 100 smaller if $B_0 \approx 0.4$ G). But as shown by the Voyager plasma experiments[20] and theoretically demonstrated by Hill[21], in the jovian magnetosphere, non-rigid co-rotation could take place even with much less magnetospheric plasma—simply as a result of angular momentum transfer from the planetary ionosphere to the Io torus plasma. However, a large degree of non-rigid co-rotation ($\Delta\Omega/\Omega \sim 50\%$) only takes place at large distances from the source region of the thermal plasma. In the vicinity of the source region, it is the mechanism of pick-up current which has more immediate effect on the rotational rate of the local magnetospheric plasma.

Following the arguments given in refs 22–24, it can be shown that a reduction of the angular velocity of the magnetosphere will be necessitated by the acceleration of the new ions of satellite or ring origin by a pick-up current system. If the total production rate of the ions is $\dot{M}$, the radial dimension of the source region ΔR, and the integrated ionospheric Pedersen conductivity Σ_p, we have

$$\frac{\Delta\Omega}{\Omega} \approx \frac{\dot{M}L^5}{4\pi B_0^2 \Sigma_p R_0 \Delta R} \qquad (5)$$

For Jupiter, $\dot{M} \approx 1.5 \times 10^4\ kg\ s^{-1}$, $L \approx 6$, $\Delta R \approx R_0$ ($= 7 \times 10^7$ m) and $\Sigma_p \approx 0.2$ S, we have $\Delta\Omega/\Omega \approx 6\%$ which is in approximate agreement with observations[25,26]. By comparison, for Uranus with $\dot{M}$ assumed to be $\leq 10^2\ kg\ s^{-1}$, $L \approx 4$, $\Delta R \approx R_0$ ($= 2.5 \times 10^7$ m) and $\Sigma_p \approx 0.01$ S we have $\Delta\Omega/\Omega \leq 1\%$. Therefore, we expect the plasma disk of Uranus within $L \approx 5$ to be rigidly co-rotating and the magnetic field topology to be hardly affected by the mass loading effect of the pick up ions (if $\dot{M} \leq 10^2\ kg\ s^{-1}$). For larger values of L (≥ 10), it is more difficult to assess the situation as there are many uncertainties. In any case, one crucial parameter in this problem is the integrated ionospheric Pedersen conductivity Σ_p which is directly related to the whole process of transport of ionization from the dayside ionosphere to the nightside hemisphere.

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Silicon and aluminium site distributions in 2:1 layered silicate clays

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Although the layer charge in 2:1 phyllosilicate minerals is known to result from the replacement of tetrahedral Si or octahedral Al, Fe and Mg by ions of lower charge, there is only limited information concerning the distribution of layer charge from X-ray crystallographic data[1–3]. Here we use ^{29}Si and ^{27}Al magic angle spinning (MAS) NMR spectroscopy to examine the site distribution of tetrahedral Si and Al in a series of synthetic trioctahedral clays with $(Si/Al)_{tetr}$ values in the range 2.74–7.69. Analysis of the ^{29}Si spectra shows that Loewenstein's rule for Al occupancy of the tetrahedral sheet is obeyed and that there is some short-range ordering for Al sites. The nature of the ordering is explained in part by the results of electrostatic potential energy calculations. In general, strong ^{27}Al resonances are observed for Al in tetrahedral sites, but the resonances due to Al in octahedral sites are considerably weaker than would be expected on the basis of chemical analysis. Consequently, no quantitative analysis of the ^{27}Al spectra is possible.

Table 1 Unit cell compositions, cation exchange capacities and X-ray diffraction data for synthetic 2:1 layered silicate clays

Compound	Unit cell composition*	$(Si/Al)_{tetr}$	CEC, mequiv. per 100 g†		d_{001} (Å)‡	
			Calculated	Observed	Air-dry	Glycerol solvated
(I)	$Na_{0.68}[Mg_{5.80}Al_{0.20}](Si_{7.08}Al_{0.92})O_{20}(OH)_4$	7.69 ± 0.23	88	91	13.1	18.0
(II)	$Na_{0.93}[Mg_{5.93}Al_{0.19}](Si_{6.64}Al_{1.36})O_{20}(OH)_4$	4.88 ± 0.08	119	134	14.2	14.6
(III)	$Na_{1.26}[Mg_{5.45}Al_{0.44}](Si_{6.51}Al_{1.49})O_{20}(OH)_4$	4.37 ± 0.12	161	170	14.0	14.5
(IV)	$Na_{1.60}[Mg_{6.17}Al_{0.08}](Si_{5.86}Al_{2.14})O_{20}(OH)_4$	2.74 ± 0.04	201	201	14.2	14.9

* The reported unit cell compositions and $(Si/Al)_{tetr}$ values are averages of five independent analytical determinations using inductively-coupled plasma emission spectroscopy. Samples were fused in lithium borate before analysis, and NBS plastic clay no. 98a was used as an analytical standard.
† Cation exchange capacities (CEC) were obtained by NH_4^+ saturated and subsequent determination of Kjeldahl nitrogen.
‡ X-ray spacings were determined for oriented film samples using Cu$K\alpha$ radiation.

Loewenstein's rule[4] states that during the isomorphous substitution of Si by Al, occupancy of adjacent tetrahedral sites by Al is avoided. An early X-ray crystallographic study[1] of Si–Al ordering in the archetypical 2:1 mica muscovite has led to a model in which the Al centres are arranged in chains, clearly violating Loewenstein's rule. More recent crystallographic studies[2,3] provide evidence for long-range ordering and separation of Al centres in the brittle mica margarite, but not in muscovite. The lack of longer-range order for muscovite does not preclude the possibility of tetrahedral Al ordering within domains, preserving Loewenstein's rule, but such short-range order is difficult to demonstrate crystallographically. Thus experiments which provide incisive information on tetrahedral site distributions are needed for the elucidation of 2:1 phyllosilicate structures.

Recent MAS–NMR studies of zeolites have shown the ^{29}Si chemical shifts to be sensitive to the number of Al ions occupying near-neighbour tetrahedral sites[5,6]. Moreover, the relative signal intensities can provide structural information which cannot be obtained by X-ray crystallography[7]. ^{27}Al MAS–NMR at high field strengths offers the possibility of providing structural information in some systems[7–9]. MAS–NMR can be equally useful for addressing the question of tetrahedral site ordering in phyllosilicates, provided that the concentration of structural paramagnetic ions is not large.

Trioctahedral clays of suitable composition and layer charge were synthesized by the hydrothermal crystallization of gels[10] at 300 °C and 100 bar over a period of 4 weeks. Table 1 provides the chemical composition and pertinent physical properties for each clay sample. Sample (I), which undergoes swelling in glycerol, may be described as a synthetic analogue of Na^+-saponite. Samples (II) and (III) carry a layer charge near the upper limit for a saponite, but they do not form a glycerol complex. Sample (IV) does not intercalate glycerol, but the layer charge is closer to the value expected for a vermiculite or mica.

Typical ^{29}Si MAS–NMR spectra are shown in Fig. 1*a* for samples (I), (III) and (IV). The relationship between the relative intensities of the ^{29}Si resonances and the $(Si/Al)_{tetr}$ values allows us to assign the lines from highest to lowest field to silicon centres in which the aluminium occupancy of the three near-neighbour tetrahedral sites is (0 Al), (1 Al) and (2 Al), respectively. None of the clays exhibits a resonance which can be attributed to Si with a near-neighbour site occupancy of (3 Al). The average chemical shifts are −95.1 ± 1.1 (0 Al), −90.1 ± 0.9 (1 Al), and −85.5 ± 0.8 p.p.m. (2 Al). It seems clear that the shifts depend mainly on the near-neighbour tetrahedral site occupancy. No second nearest-neighbour effects are observed, and there is little or no dependence of the shifts on layer charge or aluminium content of the octahedral sheet.

Representative ^{27}Al MAS–NMR spectra are illustrated in Fig. 1*b*. All of the samples exhibit an intense ^{27}Al resonance near 65 p.p.m. which is indicative of Al in a tetrahedral site[7]. Some samples (such as (IV)) also exhibit an unresolved tetrahedral Al component upfield of this resonance. Aluminium in an octahedral environment is clearly visible as a weak signal near 5 p.p.m.

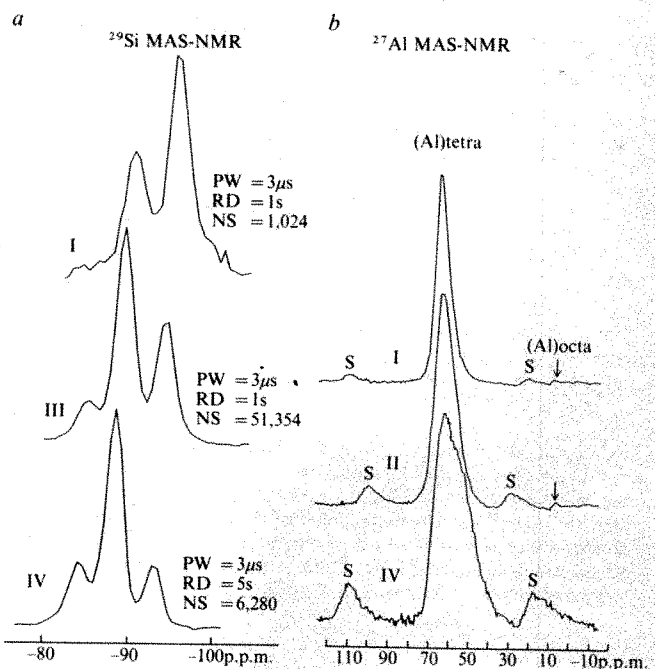


Fig. 1 *a*, ^{29}Si and *b*, ^{27}Al MAS–NMR spectra at 79.5 and 104.2 MHz, respectively, for synthetic 2:1 clays. Spinning frequencies were 4–5 kHz. Cross-polarization was not used. The pulse width (PW), relaxation delay (RD) and number of scans (NS) are provided for each ^{29}Si spectrum; the PW for a 90° flip angle was 8 μs. For the ^{27}Al spectra PW = 3 μs, RD = 40 ms, NS = 1,024. The ^{29}Si spectra were calibrated using pyrophyllite with a chemical shift of −95 p.p.m. upfield from TMS[15]. The ^{27}Al chemical shifts are relative to $Al(H_2O)_6^{3+}$. The lines labelled S are spinning sidebands.

in samples (I) and (II). However, the intensities of the octahedral Al resonances are much lower than expected from the unit cell compositions given in Table 1. Experimental pulse widths and relaxation delay times were varied over the range 3–300 μs and 0.004–2.05 s, respectively, in an unproductive attempt to improve the strength of the octahedral signal.

An analysis of the ^{29}Si MAS–NMR data, presented below, shows that our clays contain tetrahedral Al in which the near-neighbour tetrahedral sites are occupied by Si, that is, all of the tetrahedral Al is in (0 Al) environments. It is possible, therefore, that the multiple tetrahedral ^{27}Al resonances arise from differences in the occupancy of second near-neighbour tetrahedral sites. Although the octahedral Al in pyrophyllite and other 2:1 layered silicates is readily observed by ^{27}Al MAS–NMR at high magnetic field strengths, the unusually weak resonance in our samples, which are tetrahedrally charged trioctahedral structures, may be associated with a large and non-uniform electric field gradient at the octahedral sites.

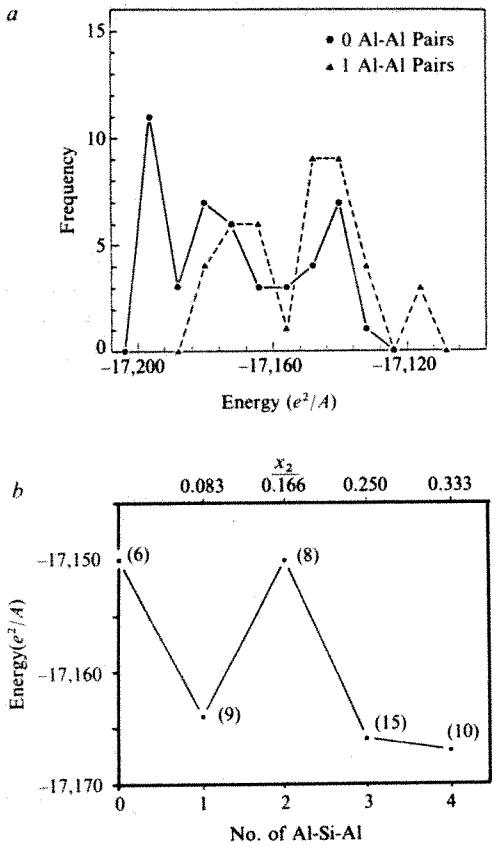


Fig. 2 *a*, The potential energy distribution obtained for 89 randomly generated mica-like structures ($x = 0.250$), grouped according to the presence of 0 (●) and 1 (▲) Al–Al linkages per unit cell [$e^2/A = 14.4$ eV]. *b*, The dependence of the average potential energies of those mica-like structures with $x_1 = 0$ on the number of Al–Si–Al linkages per unit cell. The numbers in parenthesis indicate the number of structures contributing to the average energies.

The tetrahedral site distribution in 2:1 layered silicates can be obtained from the ^{29}Si NMR intensities by an analysis similar to that made for zeolites[11]. The fraction of tetrahedral Al–Al linkages x_1, is given by $x_1 = 3x/2 - (1-x)M_1/2$, where x is the fraction of sites occupied by Al, $M_1 = (I_1 + 2I_2 + 3I_3)$, and the I_i are the normalized areas of the NMR lines due to ^{29}Si with $i = 0$, 1, 2, and 3 Al neighbours. If Loewenstein's rule holds, $x_1 = 0$, then the fraction of tetrahedral sites occupied by Al can be obtained from the NMR spectra through the expression $x = M_1/(M_1 + 3)$.

The fraction of Si sites involved in Al–Si–Al linkages, x_2, is given by the expression $x_2 = (1-x)M_2$, where $M_2 = (I_2 + 3I_3)$. For a random distribution of Si and Al in tetrahedral sites, subject to Loewenstein's rule, the probabilities of finding 0, 1, 2 or 3 Al tetrahedra adjacent to a Si can be calculated. If $(Si/Al)_{tetr} = 1/r$, the probabilities P_i, $i = 0$, 1, 2, or 3 Al, are given by the binomial distribution $P_0 = (1-r)^3$, $P_1 = 3r(1-r)^2$, $P_2 = 3r^2(1-r)$ and $P_3 = r^3$. The ^{29}Si NMR spectrum for this random distribution model consists of four lines with normalized intensities given by the P_i above.

The values of the experimental ^{29}Si-NMR line intensities and site distribution parameters defined above are given in Table 2. The values of x_1 are small and fluctuate about zero, showing that within experimental uncertainty, Loewenstein's rule is obeyed for this series of synthetic clays over the composition range $x = 0.115$–0.267. The agreement between the values of x determined by chemical analysis and by NMR analysis also is compatible with Loewenstein's rule. The experimental values of x_2 are always smaller than those predicted by the random distribution model which indicates there is some degree of short-range order in tetrahedral Al and Si sites in these structures.

Table 2 ^{29}Si MAS–NMR line intensities and tetrahedral site distribution parameters

Sample	I_0	I_1	I_2	x	x_1	x_2
(I)	0.61	0.39	—	0.115 (0.11)	0.00	0.00 (0.04)
(II)	0.52	0.44	0.04	0.170 (0.16)	0.04	0.04 (0.11)
(III)	0.35	0.55	0.10	0.186 (0.20)	−0.03	0.08 (0.12)
(IV)	0.19	0.57	0.24	0.267 (0.26)	0.02	0.18 (0.30)

The normalized integral line intensities (I_0, I_1, I_2) were obtained by fitting the observed spectra to a sum of three independent gaussian lines using a least-squares method. Values of x in parentheses were determined from the NMR line intensities, assuming Loewenstein's rule. Values of x_2 in parentheses were calculated from the random distribution model.

To understand the factors influencing the values of x_1 and x_2, particularly in the case of the mica-like sample (IV), we have carried out electrostatic potential energy calculations[12,13] on a mica structure ($x = 0.250$) with different tetrahedral Al and Si arrangements. The unit cell selected contained 16 tetrahedral sites filled by 12 Si and 4 Al. There are 1,820 different ways to fill the 16 sites. Of these, 100 were randomly selected and a crystal structure for each was derived by the DLS method[14]. Because the structural OH dipole is sensitive to cation distribution, OH was replaced by F in the model structures.

The distribution of energies for 89 of the 100 structures is shown in Fig. 2*a* where the structures are grouped according to the presence of 0 or 1 Al–Al linkages per unit cell. The remaining 11 structures, which include 2 Al–Al linkages per unit cell, have energies above $-17{,}090\ e^2/A$. There is a marked tendency for the potential energy to favour structures in which no such linkages are present (that is $x_1 = 0$). This suggests Al ions will occupy adjacent tetrahedra only rarely, in accord with Loewenstein's rule and the experimental ^{29}Si MAS–NMR results of the present work.

Figure 2*b* illustrates the dependence of the average potential energy of those structures for which $x_1 = 0$ (48 of the 89 structures) on the number of Al–Si–Al linkages per unit cell. These results show that the structures with one and four Al–Si–Al linkages (that is $x_2 = 0.083$ and 0.333) are more stable than structures containing zero and two Al–Si–Al linkages ($x_2 = 0$ and 0.166). Because of the small number of tetrahedra considered in the unit cell, the sampling of x_2 values is rather coarse. More extensive calculations with more sites per unit cell will be needed to predict accurately the most stable x_2 value.

We conclude that ^{29}Si MAS–NMR measurements on four synthetic 2:1 clays have shown that the Al tetrahedral occupancy follows Loewenstein's rule. Furthermore, by determining x_2, the number of Al–Si–Al linkages per Si, we have shown that the Al occupancy is not random, there must be short-range ordering. The calculations for a mica with $(Si/Al)_{tetr} = 3$ are consistent with this result as they show a stable configuration with an x_2 value considerably lower than that given by the random distribution model. We believe this is the first reliable demonstration of short-range ordering in 2:1 phyllosilicates.

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Nets with channels of unlimited diameter

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Three-dimensional nets and polyhedra[1,2] present an unlimited mathematical challenge, and systematic enumeration of the infinite series of four-connected nets is just developing[3–10] using selected algorithms. Several framework structures were solved and classified by invention of new nets (see refs 11–13). The four-connected nets of low density are of practical interest because their nodes may correspond with the tetrahedrally-coordinated atoms of aluminosilicate zeolites[14], some silica polymorphs[15], aluminophosphates[16,17] and other molecular sieves. The nets can be classified by circuit symbols[1–7] and coordination sequences[18,19]. Each oxygen atom of a zeolite and other tetrahedral frameworks lies near the midpoint of a branch connecting two tetrahedral nodes, and the empty volume between the close-packed oxygen atoms is important for molecular sorption. The largest ring in a natural zeolite or synthetic molecular sieve involves 12 tetrahedra[11,14], but larger rings and sphere packings of lower density are known[12,20–24]. We report here two new infinite series of four-connected three-dimensional nets which contain channels of unlimited diameter.

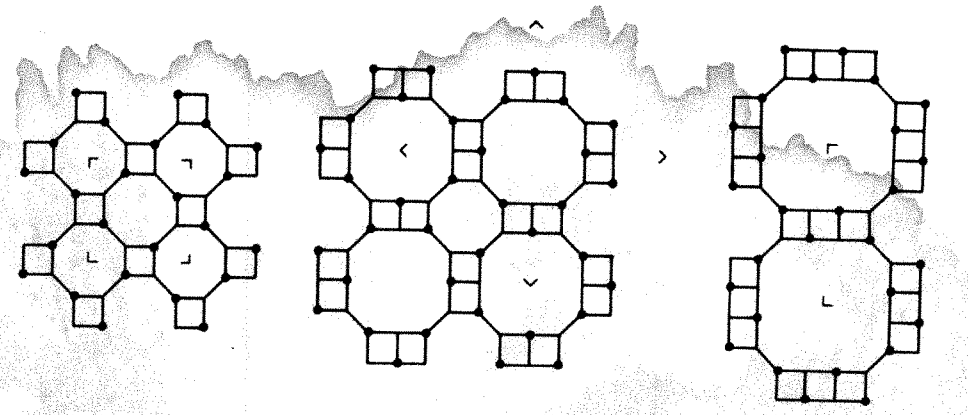
The four-connected three-dimensional net number 39 was obtained[4] from a stack of 4.8^2 three-connected two-dimensional nets by addition of alternating upward and downward branches. Figure 1*a* uses absence or presence of a dot at each three-connected node of the horizontal two-dimensional net to represent the colouring produced by the change of vertical direction of the fourth branch. Each single four-ring (or square) can be extended to an unlimited band of $(n+1)$ four-rings for which the simpler examples with $n = 1$ and 2 are shown in Fig. 1*b* and *c*. Correspondingly, the channel diameter in the three-dimensional net expands steadily as the limiting ring increases from eight nodes (*a*) to 12 (*b*), to (16) (*c*) and in general to $4(n+2)$. Relative rotation of adjacent bands of four-rings would allow an elliptical shape for the channels.

Similar alternation of vertical branches joining a stack of 4.6.12 three-connected two-dimensional nets[4] gave four-connected three-dimensional net number 81 (Fig. 2*a*) which is represented by the fifth member[17] of a series of aluminophosphate molecular sieves. Conversion of each four-ring into a band of four-rings produces a second infinite series for which the simpler examples with $n = 1$ and 2 are shown in Fig. 2*b* and *c*. As n increases, the limiting ring of the channel expands from 12 nodes for one four-ring to 18 ($n = 1$), to 24 ($n = 2$), and to $6(n+2)$ in general.

Strict alternation of the vertical branches is maintained in both series, and the highest symmetry is assumed for each value of n. Sub-series with lower symmetry, and with incongruent channels, can be developed by variation of n in different horizontal directions. The strict alternation of vertical branches converts each horizontal branch into a crankshaft chain[3]. Adjacent crankshaft chains around the surface of each near-cylindrical channel generate edge-shared hexagons (or six-rings) which form a 6^3 cylindrical net (stereoplot in Fig. 4 of ref. 17; CAN channel in Fig. 3 of ref. 25). Further infinite series with channels of unlimited diameter can be obtained by changing the sequence of vertical branches[4] and the type of vertical chain[5]; furthermore, complex cages are obtained by rotation of some branches as in net 83 of ref. 4. Another suite of infinite series can be obtained from the two-dimensional net (4.6.8)(4.8.12) found in mazzite and zeolite L. Full details will be published elsewhere.

Table 1 lists the geometrical and numerical properties of the nets in Figs 1 and 2, based on the most regular geometry and an internodal distance near 0.31 nm. It is assumed that each crankshaft chain is flexed so that the repeat distance along the chain is 0.85 nm as in aluminophosphate no. 5 (ref. 17), and that the horizontal branches of the two-dimensional net are tilted. The free diameter of each near-cylindrical channel assumes that oxygen atoms (radius 0.14 nm) are placed in positions similar to those of aluminophosphate no. 5. In a real framework, for example, of Si_2O_4 composition, the geometrical details would depend on the chemical bonding, and there is some tolerance in the distances given in Table 1. For a framework of $AlPO_4$ composition with alternation of Al and P, the topochemical symmetry is lower than the topological symmetry, as in the fifth member of the $AlPO_4$ series[17].

Direct comparison with ideal sphere packings is difficult because the latter have constant distances between adjacent spheres in contrast to the considerable variation of O–O distances in the silica polymorphs and the aluminosilicate and aluminophosphate molecular sieves. Nevertheless, it is instructive to compare the density of the present series of open nets with the least dense sphere-packings in refs 20–24. First, the density of four-connected nodes decreases systematically towards zero as n increases towards infinity for each of the series based on nets 39 and 81. For T–T = 0.31 nm, the sequence of node density per nm^3 as n increases from 0 in nets 39(n) is



(*a*) 39 (*b*) 39 (1) (*c*) 39 (2)

Fig. 1 Two-dimensional projections of the first three members of an infinite series of three-dimensional nets based on strict alternation of the vertical direction (presence or absence of dot) of a fourth branch added to each node of the three-connected 4.8^2 net. Each net is shown with the most regular geometry and highest topological symmetry. The unit cell is shown by corner marks.

Table 1 Properties of three-dimensional nets in ideal expanded shape

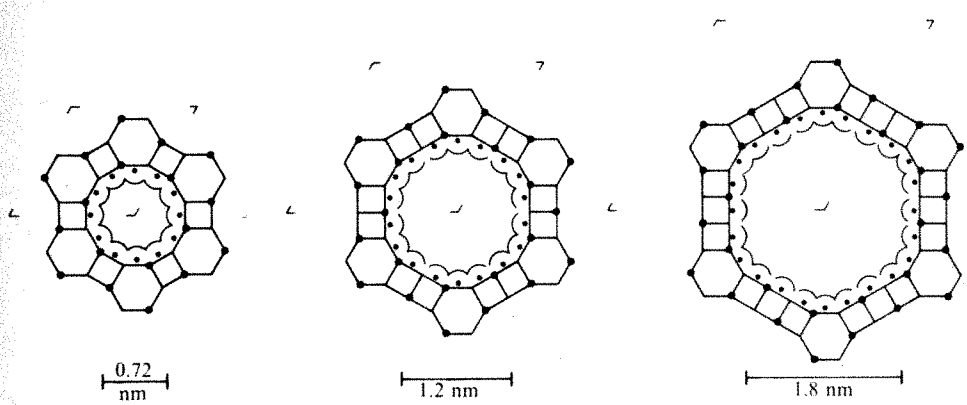
Arbitrary no.	Z_t	Circuit symbol	Space group	Z_c	a (nm)	c (nm)	Free diameter (nm)
39	8	4.6^5	I4/mcm	16	1.0_0	0.8_5	0.3
39 (1)	24	$(4^26^4)_1(4.6^5)_2$	I4/mcm	48	1.8_7	0.8_5	0.6
39 (2)	32	$(4^26^4)_1(4.6^5)_1$	P4/mcc	32	1.6_0	0.8_5	0.9
39 (3)	40	$(4^26^4)_3(4.6^5)_2$	I4/mcm	80	2.7_1	0.8_5	1.2
39 (n)	$8(2+n)$	$(4^26^4)_n(4.6^5)_2$	*	*	*	0.8_5	$0.3(n+1)$
81	24	4.6^5	$P6_3/mcc$	24	1.37†	0.85†	0.72†
81 (1)	36	$(4^26^4)_1(4.6^5)_2$	$P6_3/mcm$	36	1.8_7	0.8_5	1.2
81 (2)	48	$(4^26^4)_1(4.6^5)_1$	$P6_3/mcc$	48	2.3_7	0.8_5	1.7
81 (n)	$12(2+n)$	$(4^26^4)_{n-2}(4.6^5)_2$	‡	$12(2+n)$	$(1.37+0.5n)$	0.8_5	$(0.72+0.5n)$

For ease of reference, each sequence of nets is given the arbitrary number of the prototype from ref. 4 plus a bracketed number for the added four-rings. All estimated distances are analogous to those observed for aluminophosphate no. 5, and a range of several percentage can be obtained by varying interatomic angles. Z_t and Z_c are the number of four-connected nodes in the unique unit and the unit cell, respectively.

* n even: P4/mcc, $8(2+n)$, $1.0+0.3n$; n odd: I4/mcm, $16(2+n)$, $1.4_{75}+0.4_{25}n$.

† Observed values for aluminophosphate no. 5. All other distances depend on assumptions about bond angles.

‡ n even: $P6_3/mcc$; n odd: $P6_3/mcm$.



(*a*) 81 (*b*) 81 (1) (*c*) 81 (2)

Fig. 2 Two-dimensional projections of the first three members of an infinite series of three-dimensional nets based on strict alternation of the vertical direction (presence or absence of large dot) of a fourth branch added to each node of the three-connected 4.6.12 net. The unit cell is shown by corner marks. Small dots show approximate positions of oxygen atoms expected for occupancy of the tetrahedral nodes by Si or by alternating Al and P, and the arcs represent an atomic radius of 0.14 nm. The approximate free diameter for a molecular sieve is shown below each net.

18.8, 16.1, 14.7, 13.0, 11.7, 10.5, 9.6.... Similarly for nets 81(n), the sequence is 17.4, 14.0, 11.6.... $16.3(2+n)/(1.37+0.5n)^2$. There is also no lower limit of density for the nets[24] constructed by the Heesch–Laves[20] procedure in which a sphere is replaced by a ring of three spheres in a sphere packing.

From the viewpoint of molecular-sieve technology, the new series of nets offer an opportunity of unlimited channel diameter if synthesis can be achieved, and the products are robust. Various large ions have proved useful as templates[26] (for example, tetrapropylammonium in the synthesis of fluoride–silicalite[27]), and further exploration of large organic species, including chains and helices, should be considered. Heteropoly and isopoly complexes of the transition metals of groups 5 and 6 (ref. 28) might also be considered if suitable conditions (including *p*H) can be found for crystallization and removal of the occluded species. The existence of large channels (1.5 nm free diameter) in the mineral cacoxenite[29] should be encouraging, even though iron (III) oxygen octahedra do not provide a suitable model for tetrahedra. The 81(n) series of nets is particularly rigid, and might prove useful in engineering design. Even if the new nets have no practical value, they extend the range of mathematical theory, and provide new opportunities for artistic development.

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Quasi-liquid crystals

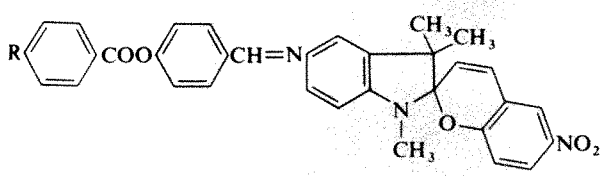
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Photo- and thermochromic conversion of spiropyrans into merocyanines[1] is used to 'engineer' new types of molecular organizates[2–6], based on the capability of the merocyanine dyes to give giant molecular stacks[7]. One type of spiropyran–merocyanine organizate, the so-called quasi-crystal, is formed when the photochromic reaction occurs in an electrostatic field. The quasi-crystals are composed of assemblies of dipolar molecular stacks covered by amorphous materials and aligned along the field[2,3].

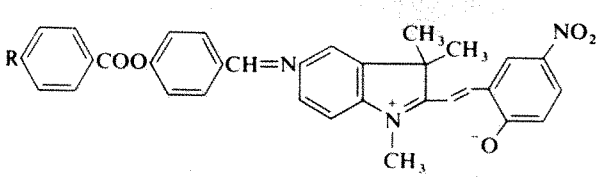
Quasi-crystals exhibit optical nonlinearity[4]. Other types of organizates occur in macromolecules bearing spiropyran side groups[5,6]. Both inter- and intramolecular stacking of merocyanine side groups have been disclosed. Here we describe a new spiropyran–merocyanine organizate obtained in spiropyrans containing mesogenic groups. These materials exhibit some features of liquid crystals (birefringence, orientation in an electric field), but have structures that are completely different from conventional liquid crystals. We propose that organic compounds with this novel form of organization be called quasi-liquid crystals (QLCs).

Spiropyrans of the general formula *A* (R = CH_3O—, *n*—$C_6H_{13}O$—, CN—, see below) were synthesized by a process that will be described elsewhere. The structures and purities (> 99%) of the spiropyrans were established by chemical analysis, NMR and mass-spectroscopy. The colour changes of these materials are associated with the following reversible reaction[1]:



A (spiropyran)

$h\nu, \Delta \rightleftarrows h\nu, \Delta$ (1)



B (merocyanine)

Crystals of these spiropyrans, obtained by slow crystallization from a suitable solvent, have sharp melting points (Table 1) and give isotropic melts.

The compounds can also be obtained as thin amorphous films by fast evaporation of the solvent from their solutions on a glass surface. In this way we prepared very viscous (grease-like) transparent films of ~0.5 μm thickness. They are metastable and remain in the amorphous state for between several hours and several days, depending on their thickness, temperature of preparation and the substituent R. The amorphous films obtained from mixtures of spiropyrans proved to be much more stable. We ascribe the formation of the amorphous films to the presence, at low concentrations, of merocyanine molecules. This merocyanine apparently acts as an impurity, retarding crystallization of the films.

Heating the amorphous films induces a change to an anisotropic structure, observable in the polarization microscope through its strong birefringent texture (Fig. 1*a*). The appearance of this texture coincides with a substantial change in the film colour, from pale yellow to bright green or greenish-blue. The change in colour is associated with a shift of the thermal equilibrium (1) to the right, with increasing temperatures. At a still higher temperature this texture disappears: above this clearing point total extinction of light is observed through cross polarizers. The temperature range in which the textured structure exists is much lower than the metling point (m.p.) of the crystals (Table 1). For example, for the spiropyran with R = CH_3O— the m.p. is 198–203 °C, while the temperature range for the birefringent texture is 50–130 °C. The material above 130 °C is an isotropic liquid; below the melting point of the crystal it is metastable. The texture disappears above 130 °C, and reappears on cooling below 130 °C. However, this process is not completely reversible due to the competitive crystallization process, which is slow at low temperatures but faster at higher temperatures. The beginning of this crystallization process is shown in Fig. 1*b*. The clusters of non-birefringent dark spots are microcrystals.

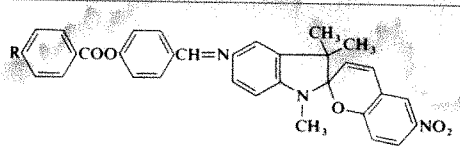
Films cooled to room temperature retain their anisotropic structure. It looks as if the mesophase is 'hidden' beneath the crystal phase and appears only when we destroy the crystal lattice by dissolving the crystalline spiropyrans. However, this is not the case, because the isotropic material obtained by heating spiropyran crystals above the melting point gives, on cooling, isotropic glass, which does not exhibit birefringence on being reheated from room temperature, that is the system does not exhibit properties of monotropic mesophases.

For orientation in an electric field a thin amorphous film was cast onto a glass slide with interdigital electrodes. The QLC were orientated in a constant electric field of $>0.5\ kV\ mm^{-1}$. For example, films of spiropyran with R = CH_3O— give at 90 °C and $E = 1.5\ kV\ mm^{-1}$ nearly homogeneous birefringence (Fig. 2). The higher the field and the temperature (below the clearing point) the better and more uniform was the orientation determined from the linear dichroism. Very thin films (<1 μm) are oriented much more slowly and less completely, probably due to an anchoring effect. Orientation in the field was also observed on the hydrophobic surface obtained by treating the glass with a solution of octadecyltrichlorosilane in bicyclohexyl.

The most remarkable effect of the electrostatic field is the stabilization of the quasi-liquid crystalline state which accompanies the orientation of the films. Spontaneous crystallization no longer occurs. In the supercooled conditions at room temperature the orientation and glass-like state were preserved without change for at least 3 months after the field was switched off. Both electron microscope results (to be published) and optical microscope observations showed the absence of crystals. However, at temperatures above the clearing point the electric field does not cause orientation but on recooling in the field the orientation is restored.

Stabilization of the oriented supercooled films allowed us to measure the absorption spectra of the QLC with polarized light (Fig. 3). The extinction coefficients of the spiropyrans (ε_{sp}) were measured in solution in benzene (Fig. 3). They are, at 370 nm, 2.23×10^4 and 1.75×10^4 for the compounds with R = CH_3O— and R = CN—, respectively. The extinction coefficients at the visible absorption maxima (ε_{mer}) of the merocyanines obtained from different spiropyrans lie in the rather narrow range $(3–5) \times 10^4\ l\ mol^{-1}\ cm^{-1}$ (refs 1, 2). Assuming these coefficients to be valid for the films we can estimate the ratio of the concentrations (C) of merocyanine and spiropyran in these films from the abosrption spectra: $C_{mer}/C_{sp} = (\varepsilon_{sp}/\varepsilon_{mer})\ (D_{mer}/D_{sp})$, where D_{mer} and D_{sp} are the absorptions of merocyanine and spiropyran, respectively. It is estimated that the extent of conversion of spiropyran into merocyanine on heating for different films is only 3–10%. A further, most surprising, fact is that the absorption spectra of the films are dichroic only in the region of the merocyanine absorption band ($\lambda_{max} \sim 600$ nm), while in the region of spiropyran absorption ($\lambda_{max} \sim 370$ nm) the degree of linear dichroism is vanishingly small. This means that only the merocyanine molecules are oriented in the electric field,

Table 1 Characteristics of spiropyrans



I R = CH_3O
II R = *n*—$C_6H_{13}O$—
III R = CN—

Compound and mixture	Melting point* (°C)	Temperature ranges of phases (°C) Amorphic → QLC	QLC → Isotropic
I	198–203	50	130
II	165–168	45	110
III	210–214	80	170
I : II = 3 : 1	—	50	135
I : III = 1 : 1	—	60	140

* Not corrected.

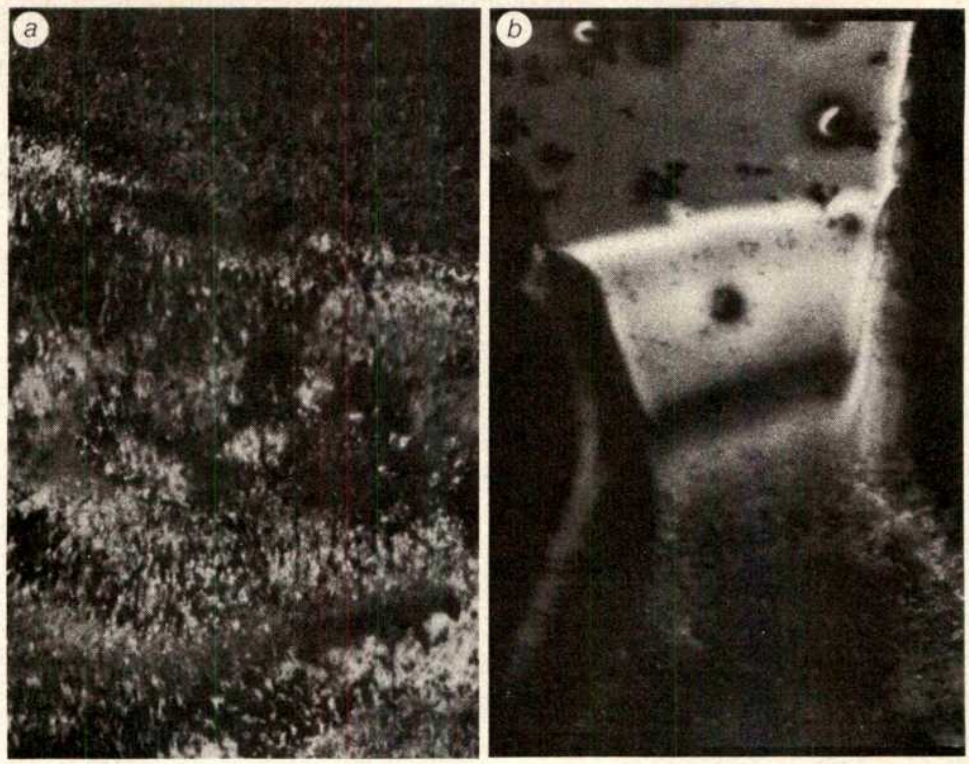


Fig. 1 Optical texture of unaligned QLC films viewed through crossed polarizers. *a*, Mixture of spiropyrans with R = CH_3O— and CN—, in the ratio 1:2, at 115 °C. *b*, Spiropyran with R = CH_3O—, at 90 °C; clusters of non-birefringent microcrystals are distinctly seen on the yellow and bluish-grey sections as shown on the front cover of this issue.

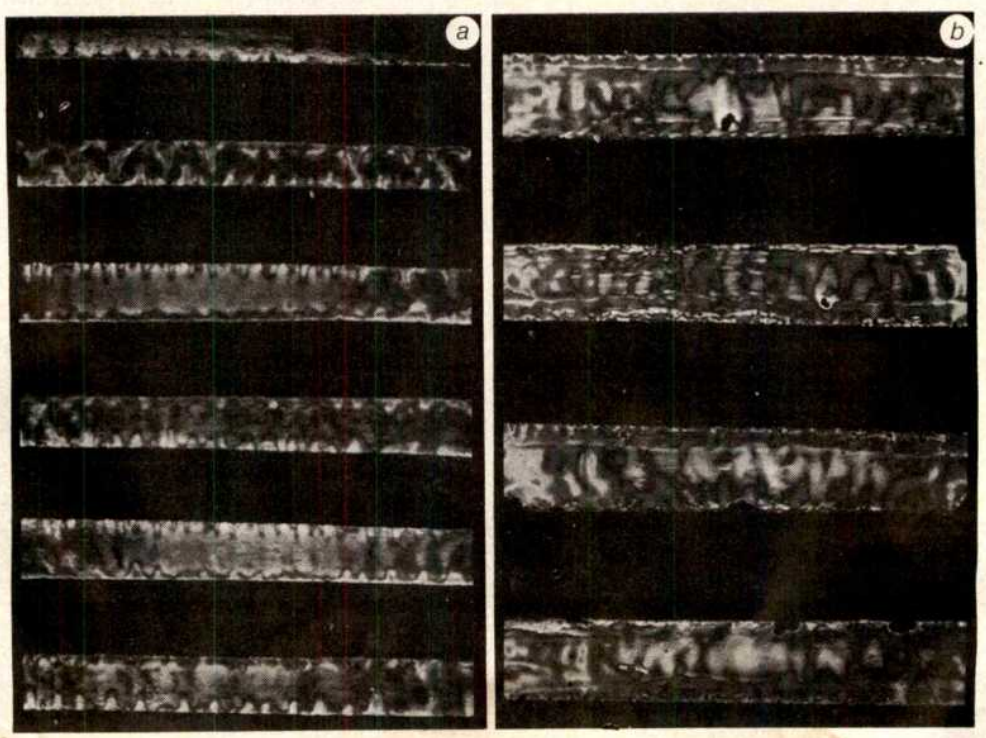


Fig. 2 Optical texture of QLC films aligned in an electrostatic field ($E = 1.5$ kV mm^{-1}, at 90 °C, distance between electrodes = 1 mm), viewed through crossed polarizers. The dark parallel stripes on the picture are areas of the interdigital electrodes. *a*, Mixture of spiropyrans with R = CH_3O— and CN—, in the ratio 1:2. *b*, Spiropyran with R = CH_3O—.

while the bulk of the material, which consists of spiropyran molecules, is unoriented.

The order parameter of the merocyanines is given by $S = (D_\parallel - D_\perp)/(2D_\perp + D_\parallel)$, where $D_\parallel$ and $D_\perp$ are, respectively, the absorptions parallel and perpendicular to the electric field. This parameter depends on the field strength, the temperature and the film thickness. For example, a 1:2 mixture of spiropyrans with R = CH_3O— and CN— gives $S \simeq 0.2$ at 90 °C and $E = 1.5$ kv mm^{-1}. The direction of the long molecular axis, coincident with the direction of maximum absorption of polarized light, is parallel to the field for all examined compounds and mixtures.

Orientation of the films in a low-frequency alternating field (~ 50 Hz) was also observed, but in this case the oriented QLC were not stable and crystallization occurred very rapidly.

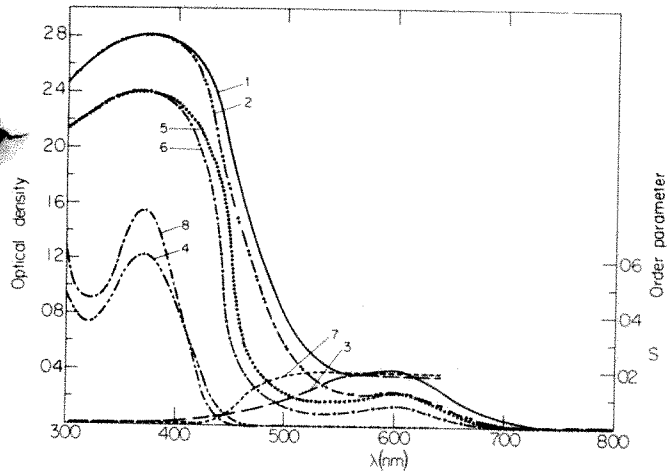


Fig. 3 1–4, Spiropryan with R = CN—: 1,2-absorption spectra of oriented QLC films, light polarized respectively parallel and perpendicular to the electric field; 3, order parameter, *S*, as a function of λ; 4, absorption spectrum in benzene solution. 5–8, As 1–4, but for spiropyran with R = CH_3O—.

Simple considerations, similar to those given in refs 2 and 3, lead us to conclude that orientation of separate merocyanine molecules is inconceivable in an electric field as weak as 1–1.5 kV mm^{-1}. Only assemblies of interacting molecules, having large net dipole moments, can be oriented in such a field. It is also inconceivable that the weak interactions, associated with the anisotropy of the polarizabilities of the mesogenic groups, and responsible for the occurrence of the liquid crystalline state of material, would keep together the merocyanine molecules in such assemblies. Indeed, the concentration of merocyanines in the isotropic spiropyran bulk is low (3–10%) and the existence of liquid crystalline domains of merocyanines in a predominantly isotropic phase is thermodynamically highly improbable.

Apparently the merocyanine molecules with the mesogenic groups somehow impose mesophase features on the bulk material: very intensive birefringence in the liquid phase, which indicates ordering of the material and characteristic temperature behaviour. These properties are not inherent in the spiropyran-merocyanines organizates that we studied previously. It is improbable that in the QLC the merocyanines or their complexes are organized in molecular stacks similar to those in quasi-crystals or in 'zipper' polymer crystals because the viscosity of the material is extremely high and stack formation by diffusion would be extremely slow. Rather, the interaction of the dipolar merocyanine molecules with the surrounding spiropyrans results in formation of small domains similar to solvating shells or to the asymmetric micelles in lyotropic systems[8]. If these domains interact with one other they may provide the material with QLC properties. On raising the temperature above the clearing point the ordering of the domains is presumably destroyed. The fact that QLCs appear only in the films cast from solution and never from spiropyran melt may indicate some kind of latent preorganization or complexation developing on evaporation of solvent.

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Sapropelic deposits in a sediment from the Guinea Basin, South Atlantic

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Until recently it has been generally thought that a stagnant water column is a prerequisite to the formation of organic-rich deposits in deep-water marine sediments, a belief best exemplified by studies on the eastern Mediterranean sapropels. Many workers have assumed that, following the hydrological isolation of the deep waters, stagnation of the eastern Mediterranean basins and the concomitant development of anoxic conditions occurred before the deposition of the sapropelic layers[1–10]. Opposing arguments suggesting a role for increased marine productivity have, however, been put forward, both specifically with respect to the eastern Mediterranean sapropels[11,12] and more generally regarding deep-water organic-rich deposits[13–15]. We report here the discovery of sapropelic layers (>0.5% organic carbon)[16] in a recent sediment core from a deep-water site in the Guinea Basin. It is thought most unlikely that stagnant conditions could prevail in the water column at this site, and preliminary analyses of the sediments suggest that the organic-rich deposits have been a direct result of increased productivity in the overlying water column, the most recent occurring about 18,000 yr ago and coinciding with the last glacial maximum.

The core (10529) was collected using a Kastenlot corer[17] equipped with a 4-m long 15 × 15 cm section barrel during RRS *Discovery* 128 cruise to the South Atlantic. The core site was on the flat abyssal plain of the Guinea Basin (4°56.8′ S 0°27.7′ E, water depth 4,735 m). The sediment core consisted of alternating marls and oozes. The oozes were grey to black in colour and distinct black-grey bands were found in each of the ooze deposits (Fig. 1).

A microscopic analysis indicated large numbers of Radiolaria and large amounts of siliceous (mainly diatom) debris in the oozes. There appeared to have been a considerable variation in the degree of carbonate dissolution throughout the core but no obvious relationship between dissolution and either the marl or ooze deposits, although particularly strong dissolution was seen at the base of the lowest ooze deposit (350 cm).

The results of a stratigraphical analysis based on coccolith distributions is shown in Fig. 2. Relative percentages of *Emiliania huxleyi* and *Gephyrocapsa* spp. have been used to determine ages. The reversal in dominance between *G. oceanica* and *E. huxleyi* is known to occur, in tropical areas, at about 85,000 yr whilst the first occurrence of *E. huxleyi* is dated at 270,000 yr (ref. 18). The peak abundance of *G. aperta* between these two dates has been recorded by Breheret[19] and Weaver[20] and it occurs in oxygen isotope stage 7. No evidence of any major sediment reworking, such as by turbidites, was found. Any such sediments would contain mixed young and old coccolith assemblages which were not present in the samples analysed in this work. The fact that the distributions closely match those found in other studies[18,20] suggests that the core is stratigraphically intact with an average sedimentation rate of 1.4 cm kyr^{-1}.

Organic carbon, carbonate and X-ray diffraction analyses[21] show the oozes to have considerably raised levels of organic carbon (Fig. 1) and opaline silica (Fig. 3*a*), but much reduced amounts of calcium carbonate. The calcium carbonate values were confirmed by X-ray emission analysis[11] for CaO (Fig. 1). The three major ooze deposite coincide with peaks in the organic carbon values with a double peak in the most recent ooze. The low carbonate values in the oozes could be caused either by dissolution or dilution of the carbonate fraction. Visually there appeared to be no consistent relationship between dissolution of carbonate tests and the ooze deposits. Furthermore plotting the organic carbon and opal values on a carbonate-free basis

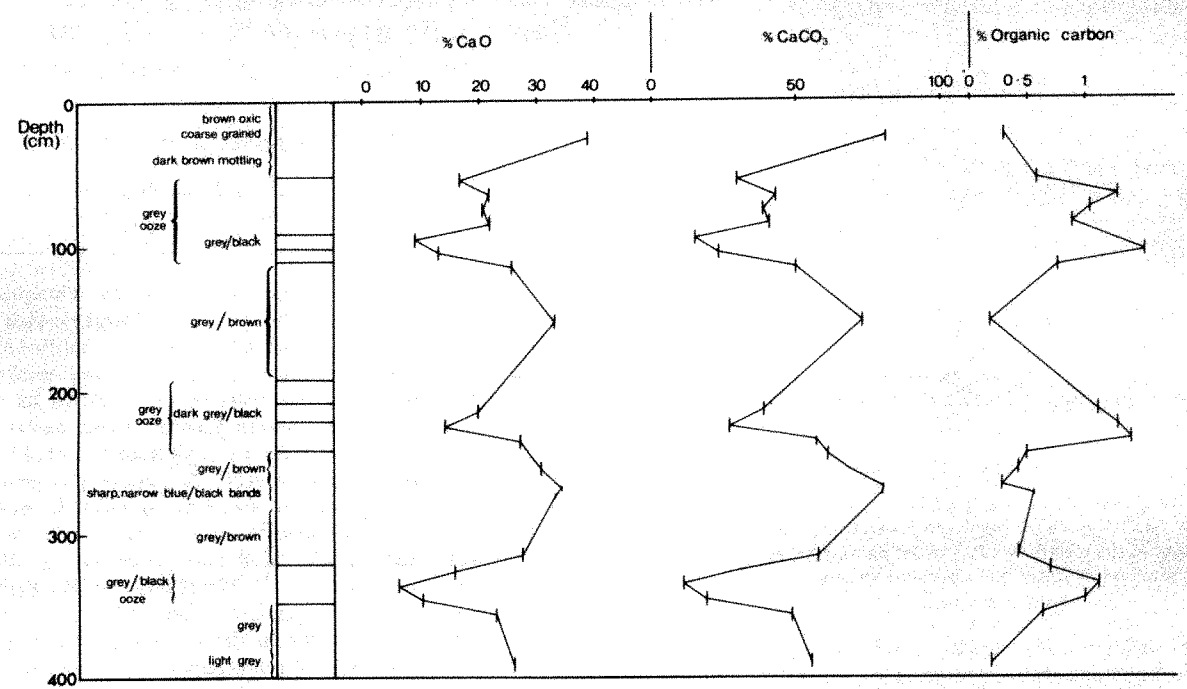


Fig. 1 Core description and content of organic carbon carbonate (% dry weight of sediment).

(Fig. 3*b*) indicates that the variation in these components are independent of variations in the carbonate content. The available evidence suggests that the increased levels in organic carbon and opaline silica reflect periods of increased inputs of biogenic silica to the sediments diluting the normal carbonate contribution.

Quartz levels in the core are generally low (<1%) but X-ray emission spectrometric analyses[11,12] indicate that there have been increased contributions of fine-grained alumino-silicate minerals to the sediment during ooze deposition (Fig. 3*a*). The origins of these inputs are at present unknown, but again there is no evidence of major post-depositional sediment transport (such as turbidites) in the core.

Some 10-cm horizons from the upper part of the core were taken for ^{14}C dating and $\delta^{13}C$ measurements (Table 1). Age data based on carbonate ^{14}C give no indications of any major contribution by turbidites in the upper 90 cm of the core, in agreement with the stratigraphical analysis (below 90 cm the age of the core is beyond the ^{14}C dating method). The ^{14}C carbonate ages give accumulation rates of between 0.8 and 3.4 cm kyr^{-1}.

Sufficient organic carbon was present in the 60–70 cm and 70–80 cm depth horizons for the determination of the component ^{14}C organic ages (Table 1). The most recent organic carbon enrichment (60–70 cm) coincides with the last (Stage 2) glacial maximum, taken here to be 14,000–19,000 yr BP. The determined organic carbon ages are considerably younger than the corresponding ^{14}C carbonate ages. This effect, though not normally to this extent, has been frequently noted[22] and has been attributed to the reworking of old carbonate particles into contemporaneous sediment[11]. In the present work, the coarseness of the sample horizon is thought to be another factor. The determined ages are an average of a 10-cm band, yet within these horizons a range of sedimentation could have occurred with the relatively steady carbonate sedimentation being periodically supplemented by shorter episodes of organic sedimentation. Turbidites are not thought to be an explanation for the discrepancy between the ^{14}C carbonate ages and ^{14}C organic ages; any turbidite action would have caused anomalies in both sets of age determinations.

The $\delta^{13}C$ measurements suggest that the organic material in the 60–70 cm and 70–80 cm horizons is derived overwhelmingly from marine planktonic sources[11,20]. This is further evidence against turbidite action; the continental shelves and slopes around the Niger delta are the most likely sources for turbidites

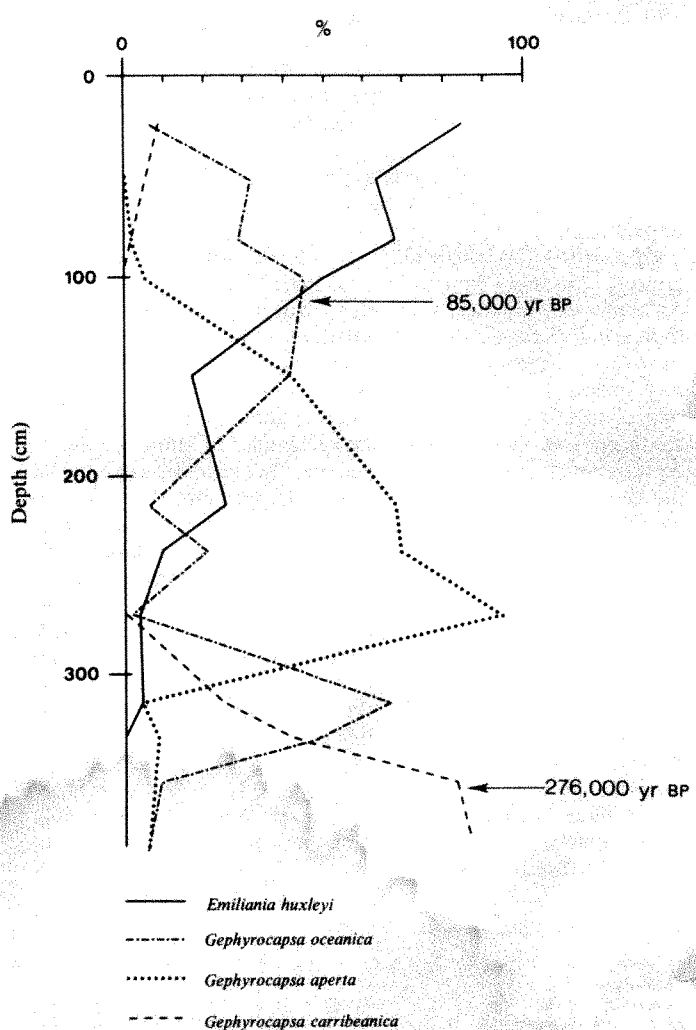


Fig. 2 Coccolith stratigraphy. The distributions suggest continuous deposition to beyond 276,000 yr. The two dates in the right hand column are derived from ref. 17.

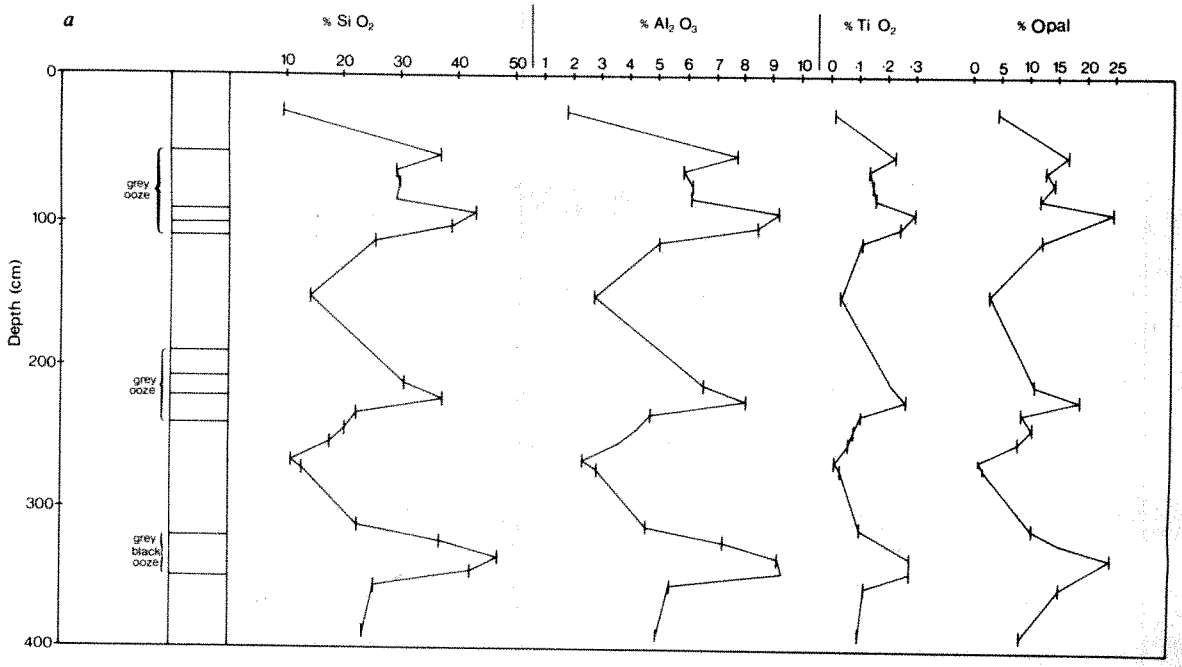
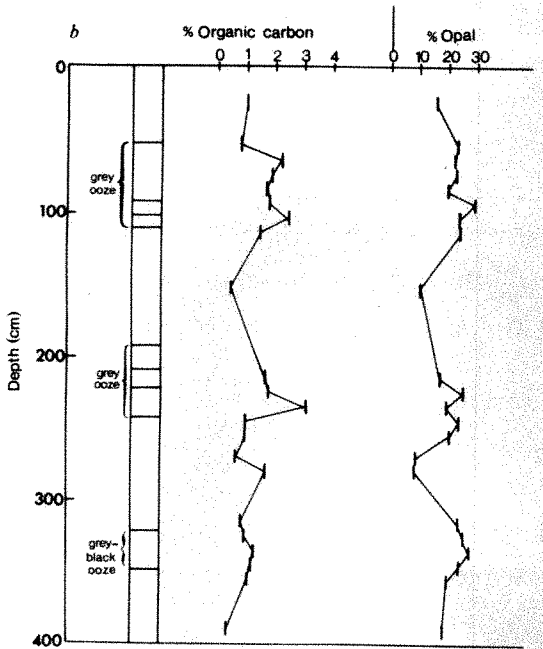


Fig. 3 *a*, Basic mineralogical composition (% dry weight of sediment). *b*, Organic carbon and opaline silica data expressed on a carbonate-free basis.



in this area and such sedimentary sites would be expected to receive a considerable contribution of organic matter from terrigenous sources resulting in greatly more negative $\delta^{13}C$ values.

Preliminary analyses on the component lipids from the dark band within the most recent ooze (97–102 cm) show a fatty acid profile dominated by C_{14}–C_{18} even chain acids (>85% total fatty acids). Long chain fatty acids (C_{26}–C_{30}), which are typical organic components of most terrigenous inputs, were only minor components (<6% total fatty acids). Such a fatty acid profile is taken to indicate that marine phytoplankton dominated the organic input during this period of the core's history with a minimal input of terrigenous organic material[23,24]. There was a considerable monounsaturated fatty acid component (11–12% total fatty acids) which is somewhat surprising for a sediment sample at least 30–40,000 yr old. We believe that for such good preservation of labile, unsaturated lipid the original organic source material must have been deposited rapidly in a relatively intact state.

Studies on some of the organic-rich oozes which underlie the present day highly productive areas of the ocean (the upwelling areas off Namibia and Peru) have indicated that in the relatively shallow continental shelf and slope areas the organic material in the sediments originates mainly from the rapid sedimentation of phytoplankton *blooms*[21,23–28]. It has been suggested that the sedimentation occurs in a series of discrete 'events', giving large pulses of biological material which sink rapidly blanketing the underlying sediment surface[25] and ensuring the burial of large quantities of the organic debris. In such conditions two processes occur which are thought to aid the preservation of the sedimented organic material. First, an increased flux of organic compounds to a sediment–water interface stimulates microbial activity with a concomitant development of anoxic conditions in the interfacial sediments. Thence bacterial degradation will be limited to all but the anaerobic species and auto-oxidative breakdown will be much reduced. Second, high molecular weight humic compounds are rapidly formed from the natural product organic molecules present in phytoplankton detrital material[26,29,30]. These high molecular weight moieties will be more resistant to chemical and biological attack than the initial natural product molecules.

Work on phytoplankton blooms occurring in experimental enclosed ecosystems has largely supported these hypotheses[29,30]. However, they only relate to geochemical processes which are

Table 1 ^{14}C dating and $\delta^{13}C$ values

Sample horizon (depth in core) (cm)	Age based on carbonate ^{14}C (yr)	Average accumulation rates (cm kyr^{-1}) based on carbonate ^{14}C	Age based on organic ^{14}C (yr)	Average accumulation rates (cm kyr^{-1}) based on organic ^{14}C	$\delta^{13}C$ (‰)
20–30	5,100±90				
		2.95			
50–60	15,260±230				
		0.80			
60–70	$27{,}720^{+630}_{-590}$		$17{,}320^{+810}_{-830}$		−20.1
		3.42		3.23	
70–80	$30{,}640^{+920}_{-830}$		$20{,}460^{+930}_{-830}$		−19.4
		1.50			
80–90	$37{,}320^{+2{,}290}_{-1780}$				
90–100	>33,720				
100–110	>37,400				

thought to occur in marine water columns of, at most, a few hundred metres.

The development of stagnant hydrographical conditions have normally been invoked as the primary process[1–10,31] for the preservation of organic material in sediments underlying water columns of several thousands of metres depth. However, peaks in the organic carbon profiles of recent deep-water sediments (2,700–3,500 m) from the eastern equatorial Pacific[15] has been taken as evidence for periods of significantly increased productivity in the area during the last glacial maximum. The sediment under present study is from a considerably deeper core site (4,735 m) in the middle of a flat abyssal plain interrupted only by a few isolated sea mounts. It is very difficult to imagine that stagnant conditions have prevailed in the overlying water columns at this site during the history of the core (~300,000 yr). Whilst conclusive evidence is not available to prove this point, there is strong evidence to support the hypothesis that the deep-water, organic-rich ooze deposits found in this core from the Guinea Basin were a direct result of periods of increased productivity in the surface layers of the overlying oceanic water column. First, the core mineralogy shows that the ooze deposits were associated with a considerable increase in the input of biogenic silica. Second, the most recent peak in organic carbon corresponds to a period of raised sedimentation. Third, the stratigraphical analysis, ^{14}C carbonate age profile, $^{13}\delta C$ measurements, quartz data and fatty acid analyses all show no major turbidite influence in the core. Fourth, the $^{13}\delta C$ and fatty acid data indicate that the organic matter in the most recent ooze deposits mostly originated in marine plankton.

The recent report of a seasonal pulse of organic detrital material to bathyal and abyssal depths being derived directly from the surface primary production[32] supports this theory.

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Isotope studies of insular phosphates explain atoll phosphatization

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Carbonate apatite[1] with the general formula $Ca_{10}(PO_4, CO_3)_6(F, OH)_2$ is a major component of the phosphate deposits that occur on numerous coral reef islands[2–6]. Such insular pohsphates are generally accepted to be the result of chemical interaction between solutions leaching seabird guano and the underlying reef limestone[2,7]. Details of this process, however, and the specific environmental conditions which promote it, are not yet fully understood[8]. We present here oxygen and carbon isotope data from carbonate apatites which elucidate the process of phosphogenesis on the Pacific atolls and the palaeoclimatic conditions in which it occurs. We suggest that there were drier climates in the tropics during the Quaternary ice ages associated with an extension of equatorial upwelling in the Pacific.

In the expectation that the oxygen and carbon isotopic composition of CO_2 sources of structural carbonate in apatite may leave a characteristic fingerprint of the depositional environment[9,10], we have initiated an exploratory study of oxygen and carbon isotope abundances of insular phosphates from coral reef islands in the tropical Pacific (Fig. 1). Samples from Ebon Island (4°35′ N, 168°43′ E) and Nauru (0°25′ S, 166°57′ E) (see Table 1) were chosen as representative of minor phosphate rock deposits on low-lying atolls and major deposits on high islands respectively. Two samples from Ocean Island (0°53′ S, 169°35′ E) and Makatea (15°50′ S, 148°13′ W) are also included to augment the representation of high oceanic islands capped by phosphates and separated by wide stretches of ocean (Table 1, Fig. 1). The phosphate from Ebon Island is typical of atoll phosphate rock, or "crust guano"[2,11]. The material consists of coral rubble, mainly coral aragonite, bound together with thinly laminated collophane (cryptocrystalline apatite) cement, thought to be a primary precipitate which forms when phosphatic solutions leached from guano first come into contact with the limestone substrate. Minor aragonite micritic cement coexists with the apatite in the fine laminae. The phosphates from the high-lying atolls are of two distinct types (see Table 1). The first are typical of the unconsolidated phosphate ore *filling* the depressions between pinnacles (karst remnants). These samples are fine to medium-size pisolitic and oolitic pellets with a markedly concretionary structure and are thought to be formed by rapid interaction of the solutions leached from the guano with the directly underlying coral-sand[7]. The second are phosphatized reef rocks *in situ*, including: (1) pure apatite cast of reef-coral where the coral carbonate skeleton has been completely removed by subsequent dissolution; (2) banded phosphate layers overlying pinnacles; (3) translucent (agate-like) phosphate called nauruite[2] occurring in depressions and cracks of the pinnacles. Materials produced from the capping of phosphate on Ocean Island, similar to the two types above, are termed by Owen[7] as incoherent and coherent phosphates respectively. We also took samples from the black phosphatic soil which covers the phosphate ore on Nauru with a thin layer. To place constraints on the timing of the apatite emplacement in the respective deposits, U-series age estimates have been used[12].

Experimental procedures were similar to those described by Kolodny and Kaplan[9], but were designed to permit separate analysis of CO_2 derived from the apatite and associated components, that is carbonate and organic matter. Accordingly, any carbonate coexisting with the apatite was selectively removed in dilute acetic acid[13]. The remaining apatite, verified by X-ray

diffraction measurements to be free of any associated carbonate, was then dissolved at 25 °C in 100% H_3PO_4 for 72 h. The isotopic fractionation of oxygen in the evolved CO_2 was assumed to be the same as calcite[9]. The organic matter residue was filtered on silicon paper and combusted at 900 °C in pre-evacuated quartz tubes in the presence of CuO and silver wire[14]. The aragonite cement from the Ebon phosphate was chipped off under a microscope and analysed separately. The CO_2 evolved from the separate fractions was analysed in a gas-source mass spectrometer for $^{18}O/^{16}O$ and/or $^{13}C/^{12}C$ isotope ratios following standard procedures[15]. The results reported according to the conventional 'δ' notation are shown in Table 1. The $\delta^{18}O$ and the $\delta^{13}C$ values from Table 1 are plotted in Fig. 2 together with those from known or likely sources for the apatite-CO_2.

These data can be interpreted as follows: The $\delta^{18}O$ value of −1.5‰ for the apatite–CO_2 of Ebon Island phosphate closely approximates the $\delta^{18}O$ value of −1.6‰ in the coexisting aragonite cement and is compatible with a CO_2 source in isotopic equilibrium with seawater[16–18]. The difference of $\delta^{13}C$ observed between the aragonite and the apatite–CO_2 (2.5‰ and 0.7‰ respectively, Table 1, Fig. 2) implies that ~8% of the CO_2 could be attributed to contamination of the inorganic carbon pool by aerobic degradation products of guano. This interpretation is supported by the $\delta^{13}C$ of the organic matter associated with the apatite (−23.5‰, Table 1) which is near that of modern guano ($\delta^{13}C = -19.5$‰) from Heron Island—a typical lagoonal platform near the southern end of the Great Barrier Reef.

Although neither Ebon nor Nauru presently support seabird colonies, the high contents (up to 9%, Table 1) of organic matter associated with the apatite from Ebon is consistent with our interpretation of a guano source, as no other mechanism would introduce such high carbon contents. The carbon-isotopic composition and the high concentration of the phosphate organic fraction thus provide direct evidence for seabird guano as the most likely and direct precursor of insular phosphates in accordance with Hutchinson's proposals[2,19].

Involvement of seawater during apatite formation on Ebon Island, implied by the oxygen isotope data, is independently supported by the relatively high fluorine content of the apatite fraction, because neither guano nor reef limestone are adequate sources for this element[12]. In fact, the wide range of fluorine content exhibited by different insular phosphates[2] has been attributed to variable exposure of insular apatites to sea spray or seawash[20] which in the case of low island deposits is not difficult to envisage.

By contrast, the distinctly light $\delta^{18}O$ and $\delta^{13}C$ yielded by the phosphates from Nauru (Table 1) may indicate apatite deposition from meteoric waters presumably following emergence of Nauru more than 200,000 yr ago. This suggestion is supported by (1) the proximity of the $\delta^{18}O$ and $\delta^{13}C$ values to the field of coral-reef limestones recrystallized in subaerial environments on Pacific islands (Fig. 2); (2) petrographic evidence which indicates that some of the coherent apatites formed as casts that preserve coral textures and that subsequently the coral dissolved presumably on subaerial exposure; and (3) geological evidence[2] which indicates that phosphatization of the atoll evidently occurred when Nauru was even more elevated than it is today. The isotopic compositions of the apatites from Ocean Island and Makatea, which are within the range of values reported for the apatites from Nauru, suggest a strong similarity between the depositional environments of these phosphate deposits. Two additional points require further discussion.

First, the extreme light $\delta^{13}C$ compositions of the apatite–CO_2 from Nauru, averaging −12‰, is consistent with a source of carbon in the meteoric water formed by roughly equal amounts of CO_2 generated by guano aerobic decay ($\delta^{13}C = -19.5$‰) and dissolution of a reef limestone substrate of $\delta^{13}C = -4.5$‰ (ref. 21). Variability in the observed $\delta^{13}C$ compositions of the apatites could reflect either departure from a ratio of 1 between the two CO_2 endmember sources and/or limestone substrates of variable $\delta^{13}C$ compositions (Fig. 2).

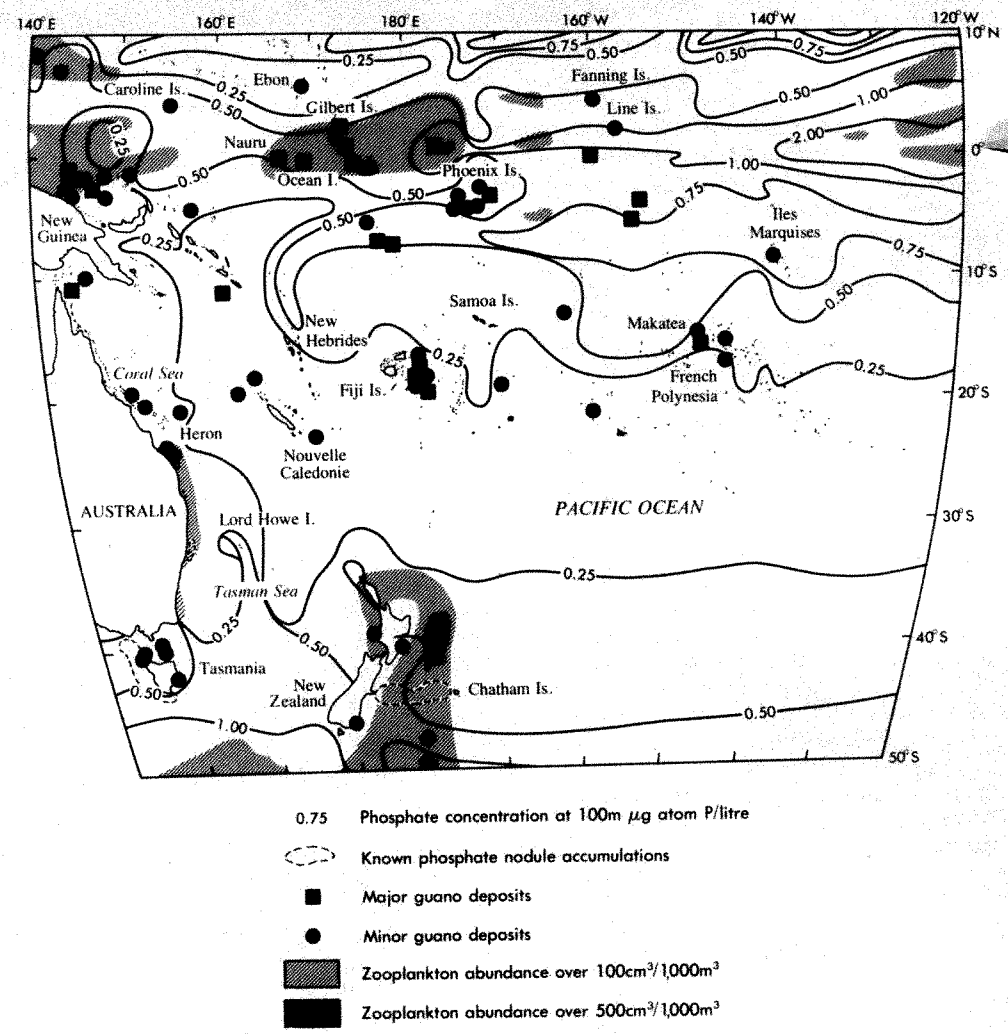
Table 1 Experimental data and stable isotope results of insular phosphates and seabird guano

Sample no. and island location	Field occurrence*	Source of CO_2 analysed	Inferred age† (yr)	Weight % CO_2	Weight % OM	$\delta^{18}O$‡ (‰ PDB)	$\delta^{13}C$‡ (‰ PDB)
Ebon							
EB 1		Total phosphatic rock		7.4		−1.72	1.51
EB 2		Aragonite cement				−1.63	2.51
EB 3	lc	Apatite	4,000 ± 300	1.0	9	−1.46	0.74
EB 4/1		Organic residue					−23.29
EB 4/2		Organic residue					−23.76
Nauru							
NR 80-5	sp	Apatite	>200,000	1.8	Trace	−6.05	−11.39
NRU-5	sp	Apatite		1.9	Trace	−6.19	−11.85
NRU-3	sp	Apatite		1.3	Trace	−5.97	−12.02
NRU-4	sp	Apatite		1.0	Trace	−5.84	−10.99
NRU-18	sp	Apatite		2.0	Trace	−6.88	−12.42
NRU-19	sp	Apatite		0.8	Trace	−6.41	−11.32
NRU-20	sp	Apatite		1.2	Trace	−6.50	−13.46
NR 3/1	rc	Apatite	>200,000	2.0	Trace	−6.03	−8.53
NR 80-9A	bl	Apatite	>200,000	3.8	Trace	−5.28	−8.50
NR 80-8	na	Apatite	>200,000	3.4	Trace	−6.28	−10.93
NRU-1	bs	Apatite		1.3		−6.10	−11.26
Ocean island							
OC-2	sp	Apatite	>200,000	1.3	Trace	−5.58	−9.39
Makatea							
MK-1	sp	Apatite	>200,000	2.2	Trace	−4.40	−10.89
Heron							
HG 1/1		Guano	Modern		98		−19.74
HG 1/2		Guano	Modern		98		−19.54

* lc, Laminated collophane; sp, unconsolidated phosphate ore of sand-sized pellets; rc, cast of reef coral; bl, banded phosphate layers on top of pinnacle; na, translucent phosphate (nauruite); bs, black phosphatic soil.

† Based on U-series age determinations of apatite fraction (ref. 12 and H.H.V., unpublished).

‡ The overall error (1σ), based on standards analysis, is estimated to be ±1‰.



0.75 Phosphate concentration at 100m μg atom P/litre

Known phosphate nodule accumulations

Major guano deposits

Minor guano deposits

Zooplankton abundance over 100cm³/1,000m³

Zooplankton abundance over 500cm³/1,000m³

Fig. 1 Distribution of guano phosphate deposits in the South Pacific Region and pertinent ocean chemistry and biology data (after ref. 32).

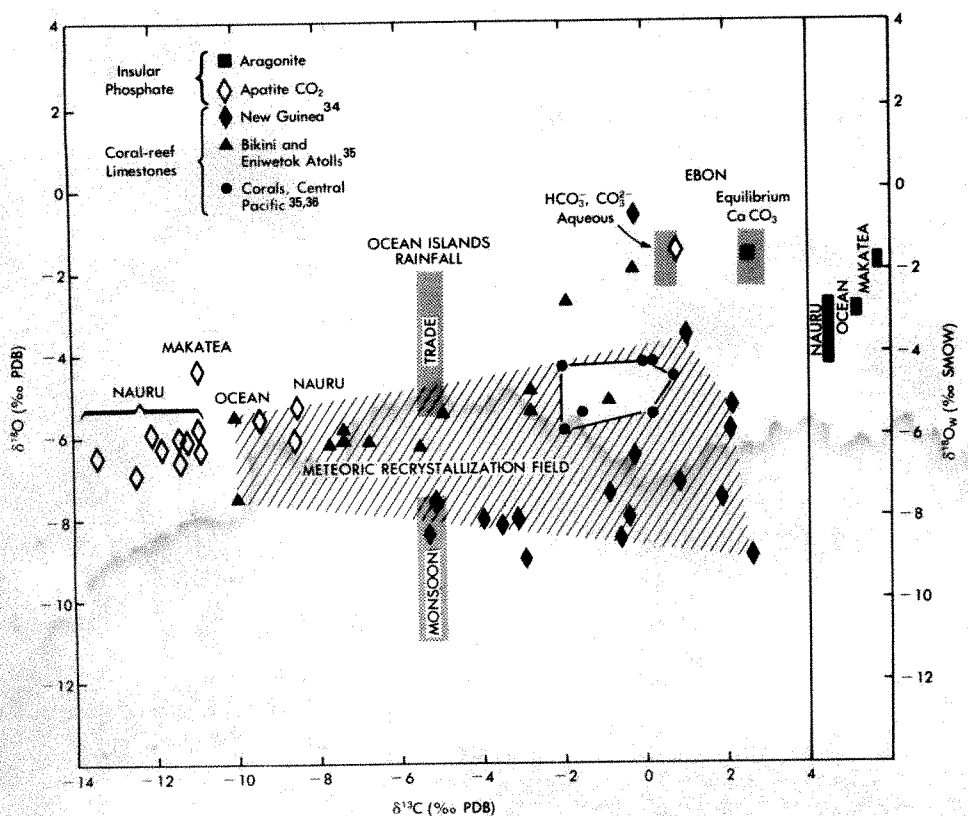


Fig. 2 $\delta^{18}O$ and $\delta^{13}C$ values of phosphatic material from low and high islands (Ebon and Nauru, Ocean Island, Makatea respectively) and the isotopic ranges of possible inorganic CO_2 sources. On the right column are the estimated $\delta^{18}O$ ranges of the palaeowaters from which the respective phosphates were deposited. Note that carbonate and phosphate $\delta^{18}O$ values are reported relative to the PDB standard while water is relative to SMOW standard. The relationship between the two reference standards is given by the expression[33]: $\delta^{18}O_{PDB} = 0.97\delta^{18}O_{SMOW} - 29.94$.

Second, groundwater which is abundant during subaerial diagenesis is a large reservoir and buffers the $\delta^{18}O$ of chemical precipitates in the vadose zone[21,22]. With one qualification to be discussed below, the narrow $\delta^{18}O$ range yielded by the subaerial phosphates reported here probably reflects the narrow $\delta^{18}O$ range of local rainfall during diagenesis[22]. In this context, the ^{18}O-enrichment of 2.5‰ observed in the apatite from Makatea relative to the apatites from Nauru (Fig. 2) is consistent with the documented $\delta^{18}O$ differences between the western and eastern tropical Pacific rainfalls[23].

If the apatite–CO_2 formed in isotopic equilibrium with the meteoric water, then the oxygen isotope composition of the phosphatizing solutions can be estimated using an ambient temperature of 28 °C, the present mean monsoonal temperature on tropical Pacific islands, and the calcite geothermometer[24]. The latter has been shown to be indistinguishable from the apatite–CO_2 geothermometer[25]. Viewed this way, $\delta^{18}O$ values of the phosphatizing solutions range between −4.3 to −2.7‰ SMOW on Nauru and Ocean Island and −1.8‰ SMOW on Makatea (Fig. 2).

Studies have shown that $\delta^{18}O$ values of groundwater reflect the isotopic composition of precipitation during the season of maximum infiltration[26]. Thus abundant monsoonal rainfalls, unusually depleted in ^{18}O relative to the trade precipitation (Fig. 2), must provide the main source of meteoric water at tropical Pacific sites affected by the monsoonal circulation[27,28]. For example, the ^{18}O-depleted meteoric water is well imprinted in the $\delta^{18}O$ compositions of late Pleistocene coral-reef limestones from New Guinea shown in Fig. 2. As isotopic modelling[29] indicates that Nauru and Ocean Island presently pertain to the same province of light $\delta^{18}O$ rainfall as New Guinea ($\delta^{18}O = -6$ to −8‰ SMOW), this reasoning would suggest that phosphates on these islands were formed from meteoric waters with ^{18}O contents appreciably different from present precipitation in the region. The apparent ^{18}O-enrichment may be attributed to the effect of monsoon failure, increased evaporation, or both, and in either case mean that major phosphate deposits of Pleistocene or earlier ages on high islands were formed in much drier climatic conditions than those now prevailing.

According to Hutchinson[2,19], the distribution pattern of islands with modern guano deposits suggests that organic productivity caused by upwelling, coupled with low rainfall, favour the net accumulation of guano and hence should provide an adequate source of phosphorus for the formation of insular phosphate deposits (Fig. 1). However, the distribution pattern of ancient insular phosphate is quite different from that of modern guano deposits and cannot readily be explained in terms of present climatic conditions.

The results of this study tentatively support Hutchinson's explanations of these differences, namely that they are related to shifts in frequency and abundance of tropical rainfall during the ice ages—factors which also affect the temporal and spatial distribution of guano phosphate deposits. Our suggestion of drier climates in the tropics during the Quaternary ice ages is quite consistent with an intensified Hadley circulation[30] and a strong development and extension of equatorial upwelling in the Pacific[31].

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An extraordinary peat-forming community on the Falkland Islands

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Most of Beauchêne Island in the South Atlantic is covered by tussac, the tussock-forming grass *Poa flabellata* (Lam.) Rasp., which has produced a deep accumulation of exceptionally dense peat during ~ 12,500 yr. The basal peat is lignitic, yet it is several hundred times too young to be a true *lignite*. During an ecological survey of the island in December 1980[1], one of us (R.I.L.S.) sampled an 11-m high peat face. The age against depth profile in the peat is consistent with a constant proportional rate of decay of $1.1–2.2 \times 10^{-4}$ yr^{-1} and a constant rate of addition of dry matter to the peat of 430–720 g m^{-2} yr^{-1}. This rate of decay is within the range recorded for peats in corresponding latitudes in the Northern Hemisphere, but the rate of addition of dry matter is about 10 times as great. This is not easy to accommodate within current hypotheses about peat formation. An unusual combination of biological, physical and chemical circumstances may be the cause. As the island is difficult to visit and no more information can be obtained in the near future, we now report these results, incomplete though they are.

Beauchêne Island[1,2] (lat. 52°54′ S, long. 59°04′ W) is the most southerly and isolated of the Falkland Islands in the South Atlantic Ocean. It is ~187 hectares in area, rises to 82 m altitude and is 60% covered by continuous closed tussac grassland. Individual plants, at a density of 600–700 per hectare, reach 3.5 m in height, including a peaty pedestal 1.0–1.5 m tall, and 2 m in diameter, and have a canopy up to 4.5 m across; a skirt of dead foliage extends from the side of the pedestal and interlaces with the skirts of adjacent plants. This is an almost truly monospecific plant community with almost no ground flora.

A further 35 hectares are occupied by dense mixed colonies of about 300,000 pairs of rockhopper penguins (*Eudyptes chrysocome*) and 160,000 pairs of black-browed albatrosses (*Diomedea melanophrys*).

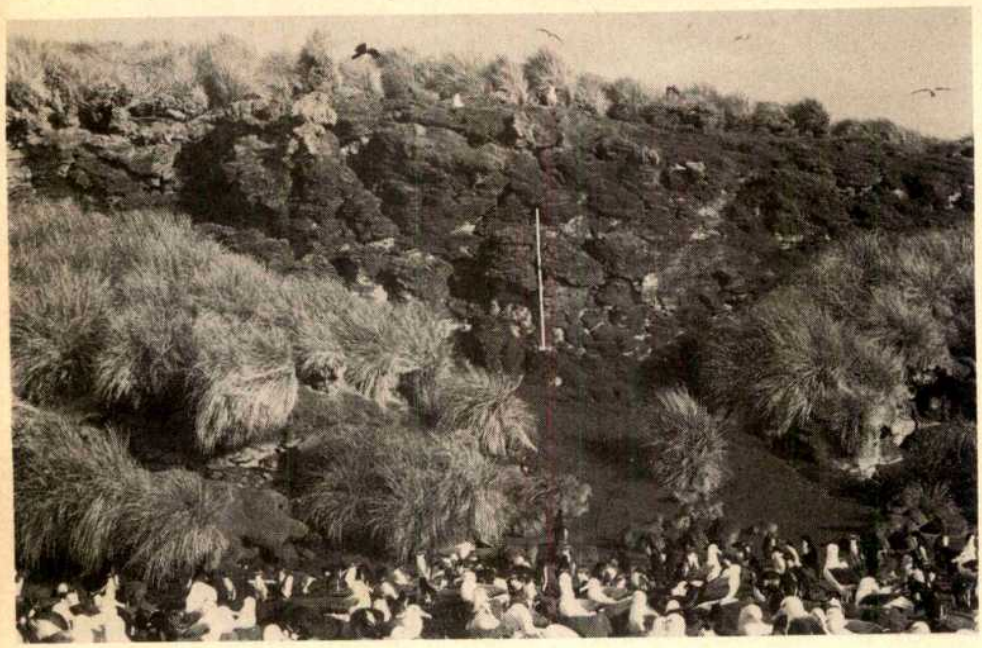


Fig. 1 An eroded cliff in the western edge of peat formed by tussac (*Poa flabellata*) grassland on Beauchêne Island in the South Atlantic. The birds in the foreground are part of a breeding colony of rockhopper penguins and black browed albatrosses. This is the site where our samples were taken. The white stake is 2.80 m long and the depth of peat from the base of the grass tussocks to the bedrock is 11.3 m. At the north-east edge of the island is a similar cliff in 5 m of peat.

The grassland grows on a deep mantle of peat composed of *Poa flabellata* litter. Along about one-third of the main bird colony the western edge of the tussac grassland is eroded into a steep and often near-vertical peat cliff (Fig. 1) along which the maximum recorded depth was 13.3 m.

Beauchêne Island has a cool temperate oceanic climate similar to, but probably cooler, drier and windier than, that of Port Stanley (Fig. 2) on the east side of East Falkland Island. It is similar to that of Tierra del Fuego. Compared with Moor House in the North Pennines of England, which has extensive blanket bog, the temperature regime is more equable with little frost, and the precipitation is much lower. Other regions of the Northern Hemisphere, such as Ireland, have extensive peat bogs with as equable a temperature regime as Beauchêne Island but even larger precipitation than Moor House. Yet others, such as continental Europe, North America and north Eurasia, have peat bogs with a much less equable temperature regime and similar precipitation.

The concentrations of inorganic species in leaves of one *Poa flabellata* plant and in three samples of surface peat from Beauchêne Island were determined[2]. These values are compared with some for North German surface peats (Table 1). The influence of sea spray is clear in the values for Na, but not in Mg. The cation exchange and chelating capacities of the Beauchêne Island peat are smaller than those of the North German peats, and this may reduce selectivity for higher valence ions. The absence of soil dust may partly account for the lower values of Ca, Fe and Al: the island rock is quartzite. Inorganic and total N are high in the Beauchêne Island peat, probably a consequence of the close proximity of so many nesting birds, but PO_4 concentrations are not very high. In the live plant there are higher concentrations (than in the peat) of NH_4 and especially of K. In general there seem to be no obvious deficiencies in the live plants, but the concentration of K in the peat may perhaps limit the activities of microorganisms, particularly at *p*H 3.7 which now prevails near the surface (Fig. 3).

The physical properties of the peat are unusual. The water content was measured on samples of peat stored for about 2 weeks in sealed polythene bags. The dry bulk density of air-dried peat was measured much later, but the results showed that during air-drying the peat had shrunk considerably. The dry bulk density of fresh peat was therefore measured indirectly by two methods. A sample was heated in water at 98 °C for 24 h. The peat was strongly coherent. Its volume was determined by displacement. The surface was blotted and the rehydrated fresh mass measured. Then the sample was put into a 'specific gravity' bottle whose mass was measured. Finally the sample was dried at 105 °C for 24 h and its mass measured. From the 'specific gravity' measurement the dry matter density, and then the volume of dry matter, were calculated. This, together with the fresh and dry mass, gave the wet volume of the rehydrated peat. The results agreed to within 5% with the direct measurements of volume. Next it was assumed that the difference in water content of the rehydrated sample and of the original wet peat gave a direct measure of the loss of volume from the peat in the fresh state and so, finally, the dry bulk density. The second method used the fact that the density of the dry matter (not the dry bulk density) changed approximately exponentially from 1.44 g cm^{-3} at 75 cm depth to 1.62 at 1,100 cm depth. With this information and the water content of the original wet peat, assumed water saturated, the minimum value of the dry bulk density was calculated. For nine samples below 125 cm deep, this calculation gave results within 3% of those obtained by the first method. This close agreement probably indicates that the dry bulk density values are sufficiently accurate and that the peat below 125 cm depth is water-saturated and hence probably anaerobic. The first method gave higher values than the second for the two topmost samples: 0.29 compared with 0.25 g cm^{-3} at 75 cm; 0.34 compared with 0.30 at 125 cm. This is consistent with some gas in the fresh peat at these depths, or with bias in the first method applied to these markedly less-coherent peats. Whatever the explanation it does not seriously affect the use of the dry bulk density values for the assessment of the peat

Table 1 Concentration of some inorganic species (μmol g^{-1}) and loss on ignition (%) on a dry mass basis

	Beauchêne Island		North Germany[9]
	Poa flabellata green leaf mid-lamina	Surface tussac peat: range of three values	Surface peat inter-quartile range
Na	160	120–150	2–10
K	490	2–10	10–60
½Ca	60	110–490	200–500
½Mg	70	83–130	160–800
⅓Fe	10	80–90	100–300
⅓Al	10	80–90	170–1,100
PO_4-P	180	6–120	30–650
NO_2+NO_3-N	0.5	0.8–5.4	<0.01–0.01
NH_4-N	240	9–27	1–15
Total − N	1,700	2,500–4,900	700–1,800
Loss on ignition	95	78–85	50–95

accumulation process (Fig. 3). In the absence of measurements of redox potential, however, we do not know the extent to which the surface layers are aerobic, and this is crucial in any attempt to understand the decay processes[3].

The bulk density of dry matter is unusually high for bog peat and the loss in mass on ignition was high (89–95%) so the high bulk density cannot be attributed to the presence of large amounts of inorganic matter. The bottom 150 cm of the peat are lignitic. Where this peat is exposed to the air it becomes black, rock-like, and breaks with a conchoidal fracture[2]. The field water content of this material is ~30% (of the dry mass) but about 1m in from the peat face the water content is ~200% and the peat has a very hard cheesy texture. True lignite is chemically altered by geothermal heat and is $>10^6$ yr old, yet ^{14}C dating shows that the lignitic peat is younger by several hundred-fold. The compressive stress at the base of a water-saturated peat may be quite small, because the effective weight is produced only by that part of the dry matter with density greater than that of water. Thus for a 10-m depth of water-saturated peat of dry bulk density 0.3 g cm^{-3} and with dry matter density 1.5 g cm^{-3}, the pressure will be $(1.5–1.0)\times0.3\times1{,}000 = 150$ g cm^{-2}.

The ^{14}C-age of 10 samples spaced down the peat profile was measured (Fig. 3) and is consistent with a rate of addition of dry matter of $p = 520–720$ (95% confidence limits) g m^{-2} yr^{-1} and of proportional rate of decay of $\alpha = 1.2\times10^{-4} – 2.2\times10^{-4}$ yr^{-1}. The asymptotic limiting depth, p/α, is 360 g cm^{-2} (there is ~330 g cm^{-2} at present). If the dry bulk density of this matter were ~0.35 g cm^{-3} then the limiting depth would be ~14 m. The position of the lowest two points may be seriously inaccurate because of the ^{14}C calibration extrapolation, and because they are in the lignitic peat which may affect the assumption of no more than two constant processes. So the topmost eight points alone were fitted too. They gave $p = 430–630$ g m^{-2} yr^{-1}, $\alpha = 1.1\times10^{-4} – 1.7\times10^{-4}$ yr^{-1}, and an asymptotic depth of 15 m.

These results are similar to those found for Fennoscandian peats[4] in that (1) depth, as cumulative mass, versus age curves appear to fit the single component, constant rate of addition, constant proportional rate of decay hypothesis surprisingly well; (2) independent of assumptions about the rate of decay, the rate of addition of dry matter has been nearly constant over thousands of years in spite of probable climatic changes; (3) the rate of decay of Beauchêne Island peat is within the range $(0.7–5)\times10^{-4}$ yr^{-1} for Fennoscandian peats; (4) the rate of addition, p, of dry matter to Beauchêne Island peat is about 10 times as great as the rate (35–78 g m^{-1} yr^{-1}) for Fennoscandian peats[4] (Fig. 3). The quoted Fennoscandian values of p are the rate of addition to the anaerobic peat and are ~10% of the net productivity of the vegation there. If the same were true on Beauchêne Island then net productivity would be about 5,500 g m^{-2} yr^{-1} which is an implausibly (but not impossibly) large value: the relative growth rate of *Poa flabellata* tillers in experiments[5,6] over the 24-week austral summer was 0.13 g g^{-1}, and a capital of 240 g m^{-2} invested with this growth rate would be sufficient to produce 5,500 g m^{-2} in 24 weeks. The dry green biomass in a site close to a seal wallow on South Georgia was 16,000 g m^{-2}.

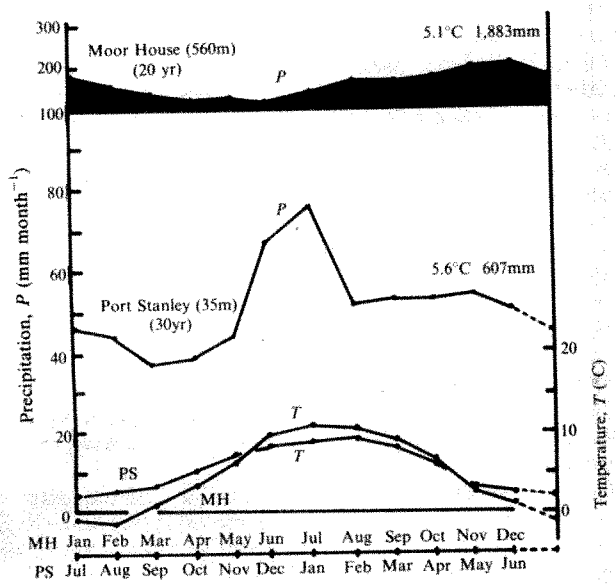


Fig. 2 Klimadiagramms (superimposed) for Port Stanley (PS), East Falkland Island and Moor House (MH), North Pennines, England.

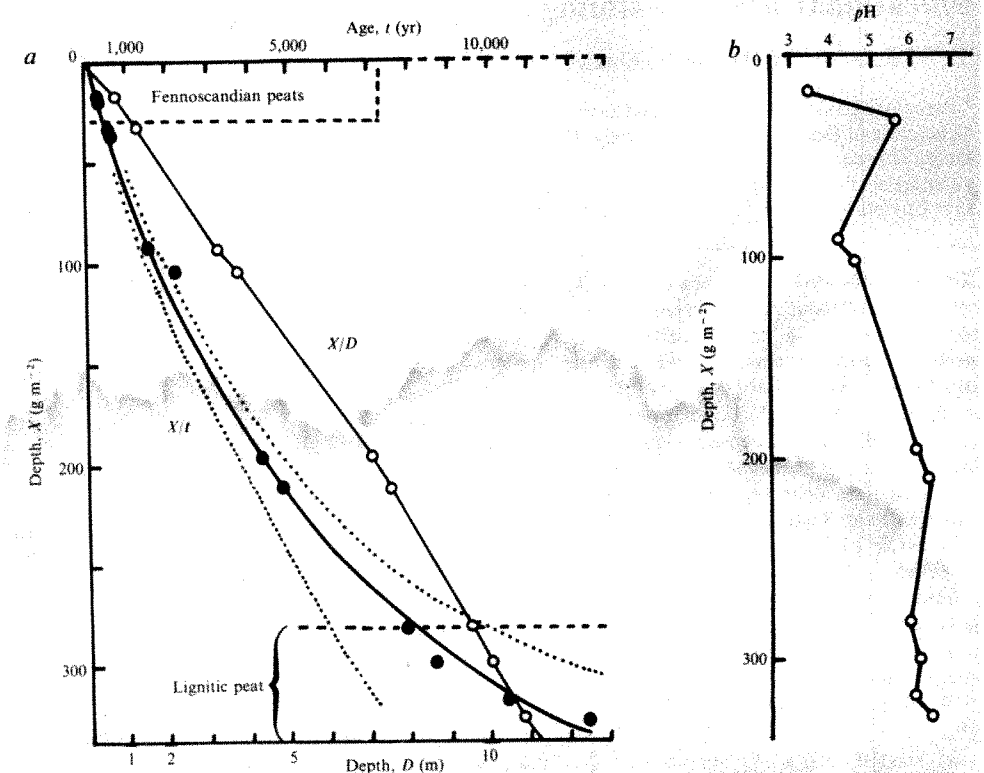


Fig. 3 *a*, Age, t, against depth as cumulative mass, X, and depth as distance, D, against depth as cumulative mass for a *Poa flabellata* peat profile on Beauchêne Island, South Atlantic. The 'true' age, t, of samples with ^{14}C age up to 6,500 yr was calculated using a dendrochronological calibration[10]. For the oldest thousand years of this calibration the deviation between ^{14}C and dendrochronological age is approximately constant and this deviation has been assumed to hold for older samples also. On this plot a constant rate, p, of addition of plant mass to the peat would give a straight line, and if there is decay in the peat a hollow curve results[3,4]. If the proportional rate of decay, α, is constant, and the peat is formed of a single component, then $X = (p/\alpha)(1-e^{-\alpha t})$. The fitted curve[4] is for $p = 620$ g m^{-2} yr^{-1} and $\alpha = 0.00017$ yr^{-1}. The estimated value of p is largely determined by the general slope of the line, and of α by the concavity. Dotted lines show 95% confidence limits. The box at the top left shows the approximate region occupied by peats in Fennoscandia[4]. *b*, *p*H against depth.

It is difficult to account for the Beauchêne Island peat. The concentration of inorganic elements in the plants reveals little about what limits the productivity of *Poa flabellata*. The species favours organic soils and is highly tolerant of sea-spray and biotic disturbance. Its luxuriance and productivity are enhanced wherever there are breeding populations of seals and seabirds. It is not known why the plant matter does not decay as rapidly as it forms. Populations of bacteria, yeasts and other fungi in the peat are high[2]. The temperature is not so low as to restrict decay in general. At the much colder sub-Antarctic South Georgia tussac peat accumulates to several metres in depth[7] yet decomposition of other organic matter proceeds quite rapidly[8]. Why then does peat accumulate on Beauchêne Island at all? And why is the rate of input to it so high at the same time as the decay rate is low? Part of the reason must be that the large input of volatilized nitrogenous compounds from the adjacent massive seabird colonies promotes rapid growth of the grass. In contrast, decomposition may be slow because of the low rainfall and high evaporation rates, caused by the almost perpetual strong winds, which together maintain a fairly dry substrate moisture regime in the surface layer of peat. In Arctic tundra peats a moisture content $<300\%$ inhibits microbial activity and decomposition rates. The water content of surface peat on Beauchêne Island is 250–350%. Potassium may also be deficient. The Beauchêne Island *Poa flabellata* peat raises fundamental issues about how peat forms, and would repay detailed study.

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Heredity and familial environment in intelligence and educational level—a sibling study

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Although it is well established that family members resemble each other in intelligence[1], the extent to which this results from either shared genes or a shared environment remains controversial[2], perhaps especially since the relevant evidence presented by Burt has been shown probably to have been fabricated[3]. The influence of heredity and familial environment may be distinguished by studying adoptees. Here we present correlations in intelligence and educational level between genetically related pairs of adult adoptees who have been reared separately, and, conversely, between genetically unrelated pairs of adult adoptees who have been reared together. We are unaware of any previous study of adults which has reported on both of these types of relationship. The results for intelligence conform closely to what would be predicted by a simple polygenic model of genetic transmission whereas those for educational attainment imply both genetic and familial environmental components.

From a Danish adoption register[4,5] we have identified four groups of sibling pairs. The first three groups comprise genetically related pairs who were adopted and reared apart in separate families: full siblings reared apart (FA, 34 pairs), maternal half-siblings reared apart (MA, 52 pairs) and paternal half-siblings reared apart (PA, 41 pairs). The fourth group comprises genetically unrelated pairs who were adopted and reared together in the same families (UT, 27 pairs). All of these adoptees were male, born between 1938 and 1947. Their median age at transfer to their adoptive homes was 5 months, and 86% had been transferred by 2 years of age.

Intelligence and education data for these adoptees were obtained from draft board records. Danish conscription law requires males, on attaining the age of 18 (or shortly thereafter, and not later than 26 years old), to appear before a draft board. A medical examination is performed, educational level (EL) is recorded on a 9-point scale from 1 = minimum years of grade school (that is, 7) to 9 = university degree, and an intelligence test, the Børge Prien Prøve (BPP)[6], is administered.

The BPP is a 45-min group test consisting of four subtests covering broad areas of cognitive functioning: letter matrices (similar to Raven's progressive matrices), verbal analogies, number series, and geometric figure analysis. Each subtest comprises approximately 20 items arranged in order of increasing difficulty. The BPP score is the total number of correct answers.

BPP and EL scores, together with height, were traced in the draft board archives for 28 FA pairs, 34 MA pairs, 30 PA pairs and 24 UT pairs.

We have also identified a fifth group comprising 73 pairs of non-adopted brothers, that is, full siblings reared together by their biological parents (FT). These pairs, for whom height and BPP and EL scores were also available, are derived from a large sample of males used in another study[7] and differ somewhat from the adoptees. They were born between 1944 and 1947, they were almost all reared in Copenhagen rather than, as were the adoptees, distributed across Denmark, and they were at least 184 cm tall.

Quantitative predictions from a familial environmental or genetic hypothesis, regarding the degree of resemblance to be expected between such pairs of adopted and non-adopted siblings on any given measure, will to some extent depend on the parameters included and on the assumptions made. Heuristically, however, the following simplification may be proposed in terms of expected intraclass correlations (ρ): a strictly environmental hypothesis would predict zero correlations among all separately reared pairs and a positive correlation between those reared together corresponding to that found in intact families, for example, usually about 0.5 for IQ[1]; a strictly genetic hypothesis of polygenic transmission, by contrast, would predict correlations corresponding to the average proportion of shared genes, that is, 0.5 for full siblings, 0.25 for half-siblings, and 0.0 for unrelated individuals. These two hypotheses are extremes, and intermediate results would indicate the presence of both environmental and genetic influences.

The most fundamental assumption of an adoption study is that genetic and familial environmental factors are independent of each other in adoptees. We have no evidence to the contrary in the present study. The genetically related adoptee sibling pairs (FA, MA and PA) were reared in familial environments which, as measured by paternal income and social class, were uncorrelated (see Table 1); correspondingly, the absence of a correlation in social class between the biological fathers of the unrelated pairs (UT) fails to suggest that the pair members are genetically correlated.

The intraclass correlations ($\hat{\rho}$) for height, BPP and EL for each of the five groups, and for the two half-sibling groups combined, are shown in Table 2. We included height on the expectation that, whatever relationships emerged for BPP and EL, this variable would show a clear pattern of heritability. Although some genetic influence is evident, it appears anomalous that the correlation for the MA group should lie, albeit non-significantly, above that for the FA group while that for the PA group should be effectively zero. This may probably be attributed to the small group sizes and necessitates caution in interpreting our other findings.

Table 1 Means and intraclass correlations ($\hat{\rho}$) for adoptive and biological fathers' income and social class

	Annual income (×1,000 kroner)					Social class				
Adoptive fathers	No. of pairs	Mean	MS_b	MS_w	$\hat{\rho}$	No. of pairs	Mean	MS_b	MS_w	$\hat{\rho}$
Full sibs apart (FA)	25	5.587	7.611	11.014	−0.22	27	2.37	2.523	2.704	−0.07
Maternal half-sibs apart (MA)	32	4.524	6.397	5.377	0.05	32	2.41	2.788	1.781	0.19
Paternal half-sibs apart (PA)	26	5.073	7.361	7.980	−0.08	29	2.62	2.523	3.690	−0.22
Half-siblings apart (MA and PA)	58	4.770	6.860	6.544	0.01	61	2.51	2.642	2.689	−0.03
Unrelated reared together (UT)	22	7.202				24	4.21			
Biological fathers										
Unrelated reared together						15	1.33	1.405	1.933	−0.23

The intraclass correlation coefficient (ρ) is estimated as $\hat{\rho} = (MS_b - cMS_w)/(MS_b + cMS_w)$ where $c = \text{d.f.}_w/(\text{d.f.}_w - 2)$. $\text{d.f.}_w = N$ pairs. Being an estimate of a ratio of variances, a negative intraclass correlation is not meaningful. The negative values in the table are those obtained from the unbiased estimator and the best estimate in the range would be $\hat{\rho} = 0$. Details of paternal income and social class were obtained from the adoption records. Income was not recorded for the biological fathers. Social class is based on a rating scale of occupation in which 0 = low and 7 = high[4]. Both income and social class are missing in some cases and the values given are derived from all available complete pairs. The mean values are for all included fathers, that is, twice the number of pairs, except for the adoptive fathers for the UT group for whom the two potentially different values, one relating to each adoption, have been averaged. Analysis of variance (ANOVA) of the adoptive fathers' means for the FA, MA, PA and UT groups yielded $F(3,184) = 4.00$, $P < 0.009$ for income, and $F(3,196) = 8.38$, $P < 0.001$ for social class. Multiple range tests (Newman–Keuls, $\alpha = 0.05$) show, for income, UT > MA and UT > PA, and, for social class, UT > FA, UT > MA, and UT > PA. Note also that we have reported elsewhere[13], for a different but comparable sample from the adoption register, that the paternal social class of adoptees, while being on average above that of the general population, does not display a smaller variance, that is, adoptees in general were raised in a representative range of social class environments.

The correlations for the BPP form a pattern conforming closely to the proposed genetic hypothesis. There are no reported studies of separately-reared adult full or half- siblings with which these results may be compared, but the only previous study of genetically unrelated adults reared together has also reported a negligible correlation (−0.03)[8].

Studies of adoptive children have usually shown a lesser degree of genetic influence, and a correspondingly stronger familial environmental influence, on intelligence[1]; perhaps both vary with age. In particular, one might expect that not all genetic effects would yet be manifested by the still-developing brain of a child[9].

The correlations for educational level in the FA, MA and PA groups suggest a lesser degree of genetic influence on this variable and, conversely, the correlation for the UT group demonstrates a marked familial environmental effect. The latter correlation may reflect influences on scholastic aspirations and diligence. The high correlation for the FT group could thus be a result of the additive effect of both genetic and familial environmental influences.

Although the intraclass correlation remains a familiar statistic, it yields less information than a direct consideration of the within- and between-group mean squares, MS_w and MS_b respectively, from which it is derived. We have therefore also fitted biometrical genetic models to MS_w and MS_b, using maximum likelihood[11,14]. In a restricted model, the total variance consists of genetic variance, G, and environmental variance, E. For each sibling pair type, total variation (σ_t^2) is partitioned into 'within' and 'between' components: $\sigma_t^2 = \sigma_w^2 + \sigma_b^2$. The expectations for these components may be derived in terms of additive genetic variance, D_R, within-family environment, E_1, and between-family environment, E_2 (ref. 11). Expected values for the observed mean squares may be readily obtained as $E(MS_w) = \sigma_w^2$ and $E(MS_b) = \sigma_w^2 + 2\sigma_b^2$ for sibships of size = 2. Table 3 gives the expected values. Assumptions in the model are: (1) no genotype–environment interaction, (2) no genotype–environment covariance, (3) $G = 1/2\ D_R$, and (4) E_1 and E_2 values are the same for all types of sibship. We have no direct test of assumption (1) in these sibships. Assumptions (2)–(4) are jointly tested by the homogeneity of the total variances across sibling types (height $F_{max}(4, 56) = 1.38$, not significant (NS); BPP $F_{max}(5, 64) = 1.59$, NS; EL $F_{max}(5, 64) = 1.64$, NS), and also by the goodness of fit of the models ($P > 0.34$). Assumption (2) can also be tested by the equality of the total variance of biological siblings reared together and the pooled variance of the siblings reared apart (BPP $F_{max}(2, 161) = 1.16$, NS; EL $F_{max}(2,161) = 1.10$, NS).

For all three variables the model provides an adequate fit with additive genetic variance significant in all cases (see Table 4). Between-family environmental variance is significant for EL

Table 2 Means and intraclass correlations ($\hat{\rho}$) for siblings' height, intelligence (BPP) and educational level (EL)

		Height (cm)				BPP				EL			
	No. of pairs	Mean	MS_b	MS_w	$\hat{\rho}$	Mean	MS_b	MS_w	$\hat{\rho}$	Mean	MS_b	MS_w	$\hat{\rho}$
Full sibs apart	28	174.7	43.87	23.66	0.27	33.38	257.50	85.16	0.47‡	3.91	7.335	3.089	0.38*
Maternal half-sibs apart	34	175.3	63.67	28.12	0.36*	32.47	122.57	91.71	0.11	3.65	2.380	3.912	−0.27
Paternal half-sibs apart	30	175.7	31.23	34.67	−0.09	35.17	154.53	77.97	0.30*	4.13	4.446	2.667	0.22
Half-sibs apart	64	175.5	48.09	31.13	0.20*	33.73	139.02	85.27	0.22*	3.88	3.413	3.328	0.00
Unrelated reared together	24	177.0	36.69	31.42	0.03	37.75	157.22	138.88	0.02	4.96	6.214	2.292	0.43†
Full sibs reared together	73	187.5	11.24	7.25		40.59	228.87	70.45	0.52§	4.95	7.225	1.404	0.67§

Being an estimate of a ratio of variances, a negative intraclass correlation is not meaningful. The negative values in the table are those obtained from the unbiased estimator (see Table 1), and the best estimate in the range would be $\hat{\rho} = 0$. The PA and MA & PA values for height are based on 29 and 63 pairs, respectively. Height was not recorded for one of the adoptees in the PA group; $\hat{\rho}$ is not calculated for the FT group because of the range restriction—all individuals were ≥184 cm. ANOVA for the four adoptee groups (FA, MA, PA and UT) yielded $F(3,228) = 4.32$, $P < 0.006$ for EL, and a multiple range test (Newman–Keuls, $\alpha = 0.05$) shows UT > FA, UT > MA and UT > PA. For BPP the overall comparison is not significant, $F(3,228) = 2.22$, $P > 0.09$, but an *a priori* comparison shows that the UT group is higher than the FA, MA and PA groups combined: $t(228) = 2.18$, $P < 0.03$.

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.005$; § $P < 0.001$.

Table 3 Expected mean squares for biometrical genetic model

		D_R	E_1	E_2
Full sibs apart	MS_w	1/4	1	1
	MS_b	3/4	1	1
Maternal half-sibs apart	MS_w	3/8	1	1
	MS_b	5/8	1	1
Paternal half-sibs apart	MS_w	3/8	1	1
	MS_b	5/8	1	1
Unrelated reared together	MS_w	1/2	1	0
	MS_b	1/2	1	2
Full sibs reared together	MS_w	1/4	1	0
	MS_b	3/4	1	2

D_R, Additive genetic variance; E_1, within-family environmental variance; E_2, between-family environmental variance.

Table 4 Biometrical genetic models

Parameter	Estimate ±s.e.	z	Model fit d.f.	χ^2	P
Height model					
D_R	53.349 ± 21.73	2.45*			
E_1	7.321 ± 12.49	0.59			
E_2	3.259 ± 7.62	0.43	5	5.2857	0.3820
BPP model					
D_R	256.20 ± 66.23	3.87†			
E_1	3.95 ± 19.97	0.20			
E_2	6.15 ± 15.99	0.38	7	4.6248	0.7056
EL model					
D_R	4.162 ± 1.818	2.29*			
E_1	0.324 ± 0.537	0.60			
E_2	1.655 ± 0.478	3.46†	7	7.8416	0.3468

* $P < 0.05$; † $P < 0.001$.

only. Biometrical genetic modelling allows estimates of the effects of dominance deviation and assortative mating[10,11,14]; the results of the goodness-of-fit tests imply non-significance for both parameters in these data.

Some degree of familial environmental effect on intelligence and educational level can be detected in the means of the four adoptee groups. The adoptees in the UT group were reared in homes that were significantly wealthier and of a higher social class than those in the other three adoptee groups (see Table 1). Parents adopting more than one child were better situated than adoptive parents in general; this has elevated the intelligence and educational level of their adoptive children. Compared with the FA, MA and PA groups, the UT group adoptees have a significantly higher BPP and EL, the latter effect being more marked than the former (see Table 2). The mean differences in EL and BPP between the adoptees taken together (FA, MA, PA and UT) and the non-adopted siblings (FT) are not easily interpreted as either or both groups may deviate from the general population, and genetic or environmental differences may be involved.

The correlation between BPP and EL is 0.65 for all adoptees and 0.71 for the non-adopted siblings; this replicates the strong relationship usually found between intelligence and educational level[12]. These two variables are generally thought to interact with each other. As our findings suggest stronger genetic effects on intelligence than on educational level, it could be that genetic influences on educational level are indirect and operate primarily through genetic effects on intelligence. Conversely, however, the effects of familial environment on educational level appear stronger than the effects on intelligence. Therefore, the effect of familial environment on intelligence might be the indirect result of a direct influence of education itself on intelligence. In view of the comparatively small sample sizes used in the present study, and the (perhaps consequent) paradoxical findings for height, this interpretation must only be tentative.

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Vertical disparity pooling and the induced effect

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If the image received by one eye is vertically magnified by a small amount then an illusory tilt is perceived around the vertical axis through the fixation point. This is known as the induced effect[1] and it can be explained by a recent computational theory of binocular vision[2–4] which treats it as a side-effect of the use of vertical disparities to recover information about the distance to the fixation point and the angle of gaze. We have investigated the consequences of introducing vertical magnifications of some parts of a scene and not others and report here that there is a simple linear relationship between the size of the induced effect and the average vertical magnification. This suggests that a pooling strategy is adopted in the measurement of vertical disparities, a result which fits in well with expectations of the theory.

The computational theory of binocular vision[2–4], which demonstrates how vertical disparities can be used to discover the distance to the fixation point (d) and the angle of gaze (g) with which the fixation point is being viewed, is introduced in ref. 5. One prediction of the theory is that vertical disparities produced by a monocular vertical magnification will lead the system to compute an incorrect angle of gaze[3]. Since this inappropriate value of g is then used to scale the horizontal disparities an erroneous left/right tilt of the entire field of view will result. This explanation of the induced effect will be called the 'd/g hypothesis'.

We have investigated how vertical disparity information is integrated from various parts of the field of view. We wished to know whether a mixture of vertical disparities that was incoherent, in the sense that it would not arise in normal viewing of natural scenes, would destroy the induced effect because it would then be difficult to compute the correct d and g from the conflicting information[3]. Alternatively, since the vertical disparities observed in normal viewing are quite small and perhaps difficult to measure accurately, the visual system might adopt a pooling strategy that would be reflected in an orderly relationship between the average magnification and the size of the induced effect created.

The experimental stimulus was an array of dots seen by binocular fusion of a pair of computer-controlled high-resolution x–y displays (Hewlett-Packard 1332A) that formed

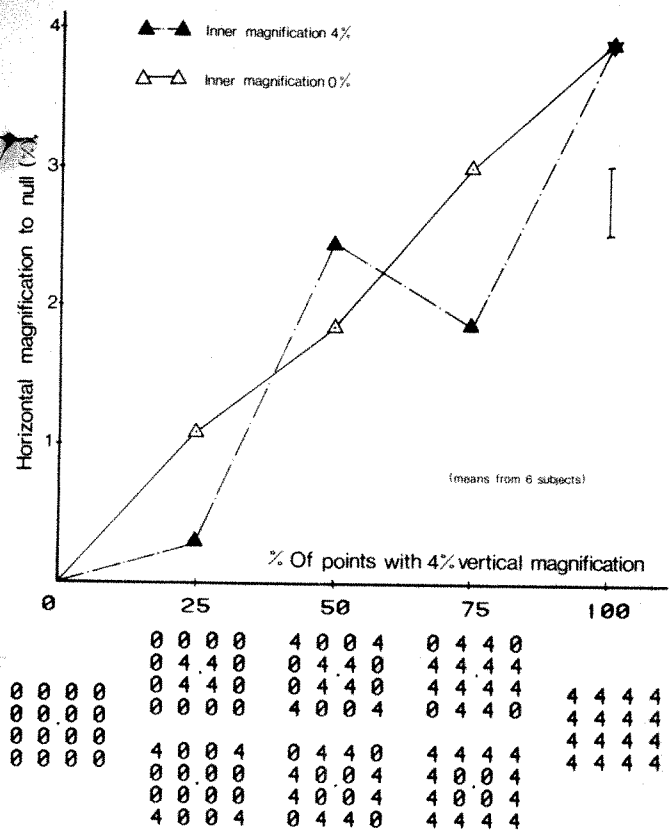


Fig. 1 Relationship between the size of the induced effect and the percentage of dots adjusted in position by a 4% monocular vertical magnification. Diagrams below the abscissa show the spatial distribution of dots whose positions were adjusted by 0% and 4% vertical magnifications. For the purposes of this graph (but not for the statistical analyses) each subject's mean setting for the 0% condition, which measured his subjective fronto-parallel plane, was subtracted from all points so that the plots illustrate directly the size of the induced effects. Solid triangles show means for the conditions where the four innermost dot positions were magnified (top row of diagrams); open triangles show means for conditions where the four innermost dot positions were unaltered (bottom row). The vertical bar on the right of the figure shows the mean standard error.

Methods: The dots had diameters of ~2.6 arc min, luminance of ~13.1 cd m^{-1} and could be positioned to an accuracy of ±0.2 arc min. They were arranged in a regular lattice except that the position of each dot was randomly displaced by up to ±8.5 arc min in both the x and y axis to avoid easily detectable shape cues associated with the various stimulus conditions. The overall size of the array was 7.2×7.2 deg visual angle.

the left and right fields of a Wheatstone stereoscope. Induced effects were created by altering the positions of selected dots in the right field. This simulates the effect of a meridional size lens, and gives a monocular magnification of the vertical projections of dots from the horizontal meridian through the fixation point[1]. Measurement of the induced effect was by a nulling technique which cancelled the induced effect with an opposite tilt illusion called the 'geometric effect'. The latter is also an illusory tilt around the vertical axis, but in the opposite direction, and is produced when the image received by only one eye is horizontally expanded by a small amount. This illusion was produced by simulating the effect of a meridional size lens with its axis vertical.

The subject was seated in a completely dark room, with his head position fixed by a forehead and chin rest. He saw about 40 cm away from him at eye level a 4×4 array of small bright dots with a fixation point at its centre (see Fig. 1 for details). At the start of each trial the display possessed some randomly-sized left/right tilt by virtue of the induced effect being measured having superimposed upon it an initial geometric effect, randomly selected within the range ±4.5%. Subjects were not informed of the source of the tilt they saw at any one time, whether it was induced effect, geometric effect or a mixture of the two. Their task was to press a pair of buttons until the array of dots appeared to lie in a fronto-parallel plane through the fixation point. A press on one button increased the nulling horizontal magnification by 0.5%, the other decreased it by 0.5%. Subjects were allowed to track to and fro over their null region before deciding on a setting, but training sessions ensured that they were able to make each setting within ~15 s.

Six subjects were used, each providing 10 settings per condition but with the largest and smallest eliminated to reduce experimental noise. Each block of 10 settings required about 3–5 min and to reduce fatigue successive blocks were separated by breaks of ~5 min during which subjects left the experimental room. A fuller account of the experimental design will be presented elsewhere[6].

All eight experimental stimuli are shown schematically below the abscissa in Fig. 1, where 0s and 4s indicate dots whose positions were adjusted by 0% and 4% magnification respectively. These 'mixed magnification' stimuli comprised two groups, distinguished by whether the vertical projections of the inner four dots of the array were 4% magnified or left unaltered. The two types permitted a preliminary exploration of the importance of the distribution of magnified points.

The results shown in Fig. 1 indicate that the induced effect created with uniform 4% vertical magnification was nulled by an opposing horizontal magnification of almost exactly equal size. This result replicates a well attested result regarding the relationship between induced and geometric effects[1] and suggests that the novel technique used here to measure the induced effect was satisfactory. The two types of mixed magnification stimuli did not produce significantly different tilts ($F[1, 5] = 1.258$, not significant), nor was there a significant interaction between that factor and the percentage of magnified points ($F[2, 10] = 3.036$, not significant). On the other hand, the magnitude of the induced effect increased with the percentage of magnified points ($F[4, 20] = 13.63$, $P < 0.0001$), with a trend analysis incorporating data pooled over the two types of mixed magnification stimuli showing only the linear trend to be significant ($F[1, 20] = 91.916$, $P < 0.0001$); slope of linear regression = 0.924).

This simple linear relationship suggests that the size of the induced effect is determined by the 'average vertical magnification' and implies that vertical disparity information from different parts of the field of view is pooled. The similarity between the two types of mixed magnification stimuli indicates that there is no significant difference in weighting between relatively central regions and peripheral ones. Pooling fits in well with the d/g hypothesis because viewing system parameters are intrinsically whole-field in character: as only one d and one g can exist at any one time, it makes sense to pool information about them, particularly if that information is delivered by vertical disparities which are inevitably small. The fact that vertical disparities are scaled by retinal eccentricity but unaffected by local variations in depth[3] would be compatible with a pooling mechanism that allows data to be pooled over relatively large areas.

There are a variety of ways in which vertical disparity information might be pooled. These might be divided into two broad classes distinguished by whether locally computed values of d and g are averaged or whether vertical disparity information is pooled before the computation of d and g. Whatever the particular scheme adopted by the human visual system, the result reported here suggests that an orderly pooling of vertical disparity information does occur.

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Antibodies to heavy neurofilament subunit detect a subpopulation of damaged ganglion cells in retina

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Neurofilaments (NFs) consist of three protein subunits with apparent molecular weights of 68,000 (68K), 145K and 200K, which are found closely associated in most but not all locations in the nervous system[1–4]. One of these exceptions is the inner retina of the mouse, where antibodies to 145K NFs label large ganglion cells throughout the extent of the cells, while antibodies to 200K NFs label only more distal portions of the optic axons but usually fail to label the ganglion cell somata and proximal axons[5,6]. Very rarely, however, and more often in old mice, anti-200K NF antibodies do label a ganglion cell completely[5]. To determine whether these rare, completely labelled cells reflect a pathological alteration, we cut the optic axons, and report here that after a few days some of the axotomized cells could be labelled completely, in a Golgi-like fashion, by anti-200K NF antibodies. These cells seem to represent the population that forms the projection to the bulk of the lateral geniculate nucleus, as suggested by their size, distribution and projection pattern. Hence, antibodies to the heavy NF subunit in combination with lesions may allow selective retrograde tracing of a subpopulation of ganglion cells, and such antibodies can be used to detect damage in NF-rich neurones at a very early stage, long before they eventually degenerate.

Neurones respond to axonal injury with the retrograde cell reaction—chromatolysis and displacement of the nucleus—which may be followed by recovery, or, for centrally projecting neurones in mammals, often by degeneration of the entire cell[7,8]. Lesions of optic axons cause retrograde degeneration of retinal ganglion cells in mammals, which in adults is a rather slow process that may take months to years[9,10]. The change in affinity to NF antibodies in response to axotomy described here for the retina represents another feature of the retrograde reaction that is pronounced only in the largest ganglion cells. A similar abnormal neurofilament labelling has been observed in lesioned peripherally projecting neurones, where it was attributed to neurofibrillar hypertrophy[11]. The abnormality in the retina, however, reflects primarily an antigenic change rather than an increase in neurofilaments.

The binocular visual field in the mouse is largely located above the head, and is seen by the lower temporal retina. Some of the ganglion cells in this retinal region project to the brain on the same side, where they join the projection from the opposite eye, a convergence that forms the substrate for binocular vision[12]. Thus, when the optic tract on one side is lesioned, the effect of axotomy can be compared with the normal condition in the same retina, ipsilateral to the lesion. In the nasal retina, representing normal conditions (Fig. 1*b*), optic axons labelled by anti-200K NF antibodies can be followed from the optic disk for only a limited distance; ganglion cell somata and proximal stretches of axons remain invisible. In contrast, in the lesioned cells in the lower temporal retina (Fig. 1*a*) the reaction with the antibodies extends to the proximal axons, cell somata and dendrites, as also seen at higher power in Fig. 2. The labelled cell bodies were large (17.2 ± 1.8 μm mean diameter, $n = 207$), rather regularly spaced, and were probably identical to the large, NF-rich ganglion cells seen in normal mouse retina using methods that detect NF subunits of lower molecular weight[6,13,14]. Camera lucida drawings of all labelled ganglion cells ipsilateral to the optic tract lesion (Fig. 3*a*) showed about 300 cells (312 ± 14, $n = 6$), representing about one-third of all ipsilaterally projecting cells in the mouse[12].

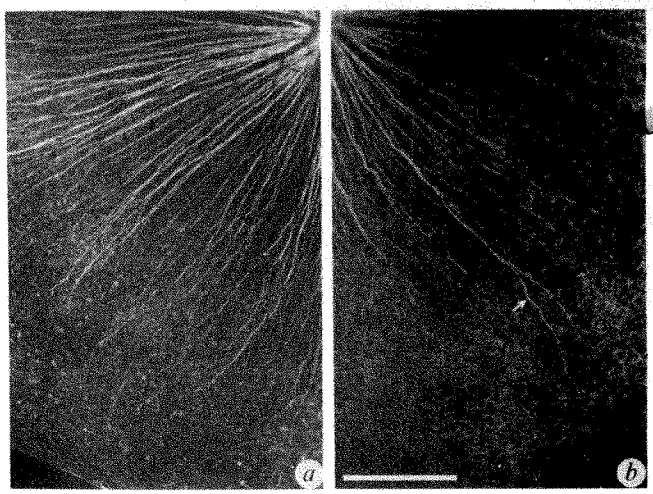


Fig. 1 Whole-mount of mouse retina showing effect of axotomy. A young adult C57BL/6J mouse was anaesthetized with Nembutal, and one optic tract was exposed and sectioned. After 10 days the mouse was perfused with paraformaldehyde and the retinas were reacted with an anti-200K NF antibody; here RT97 was applied[21], which gave results similar to the anti-200K NF antibodies R3 (ref. 5), 06-17 (ref. 22), 4.3F9 and 2.2F3 (ref. 23) used in other preparations. Antibody binding was visualized with a fluorescein isothiocyanate-labelled secondary antibody (Boehringer-Mannheim). Shown here are sectors from the retina ipsilateral to the optic tract lesion. *a* Includes part of the retinal representation of the binocular visual field which sends a projection to the ipsilateral side of the brain. *b*, Retinal sector corresponding to the monocular field represented only in the contralateral brain; hence the ganglion cells here were not affected by the optic tract lesion. The position of the optic disk, just above the upper margin in both views, can be inferred from the converging optic axons; the ciliary edge of the retina is visible in the lower corners of the figure. The textured background of the entire retina is due to labelling of the NF-rich axonless horizontal cells in the outer retina[24]. Note that in the unaffected retinal sector (*b*) the labelling of optic axons increases in intensity towards the optic disk, and even the longest axon segments visible cannot be traced much further than about half-way through the extent of the retina. In the sector containing axotomized ganglion cells (*a*), however, some optic axons can be followed all the way to their cell bodies, which are barely visible at this magnification in the peripheral retina. The increasing affinity of anti-200K NF antibodies for more distal portions of axons in normal conditions is further illustrated by the fibre indicated by an arrow in *b*: for this fibre the segment in the retina was the distal portion of the axon; it entered the retina through the optic disk and gave off branches on its course, a characteristic of efferent fibres (called efferent with respect to the brain[25]). It is unknown where the cell bodies of such efferent fibres in the mouse are located. Scale bar 500 μm.

In contrast to the limited retinal region giving rise to the ipsilateral retinofugal projection, contralaterally projecting ganglion cells are known to originate from the entire retina, and to outnumber the ipsilateral projection by about 60 to 1 in the mouse[12]. After the induction of optic tract lesions, however, the ganglion cells that assumed Golgi-like affinity to 200 K NF antibodies in the retina contralateral to the lesion formed a small subpopulation: about 1,000 cells were labelled, representing ~2% of the contralateral projection. The labelled cells were large and regularly distributed throughout the retina except for the lower temporal region which consistently contained only a scattered few labelled cells; this sparsely labelled region corresponded to the ipsilaterally projecting region in the other retina (Fig. 3*b*). Hence, a lesion of optic axons caused abnormal affinity for anti-200K NF antibodies in a subpopulation of ganglion cells which cover the retina more or less evenly and which have a line of decussation in their projections to one or the other side of the brain.

Such a pattern of projections is compatible with retinal inputs to the geniculo-cortical system but not to the optic tectum, as

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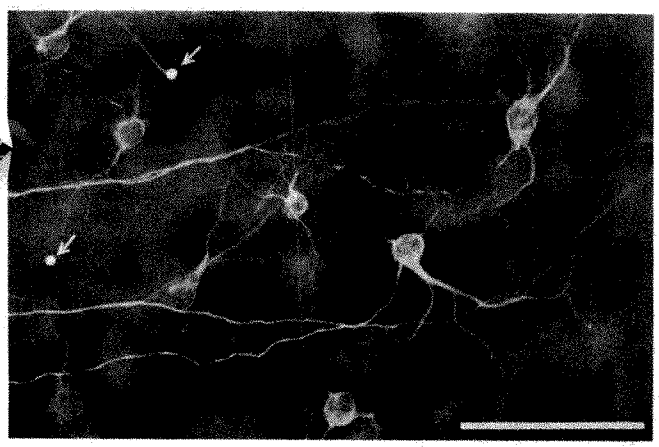


Fig. 2 Ganglion cells in retina ipsilateral to an optic tract lesion made 15 days previously, labelled for 200K neurofilaments with RT97 (ref. 21). These cells were the largest ganglion cells and formed about one-third of the entire ipsilateral projection[12]. Arrows indicate neurofilamentous clubs[8] that may represent the axons of some of the smaller axotomized ganglion cells. Such neurofilamentous clubs appeared a few days after the optic tract lesion and increased in frequency with time. They are also visible as bright specks in Fig. 1*a*. Scale bar, 100 μm.

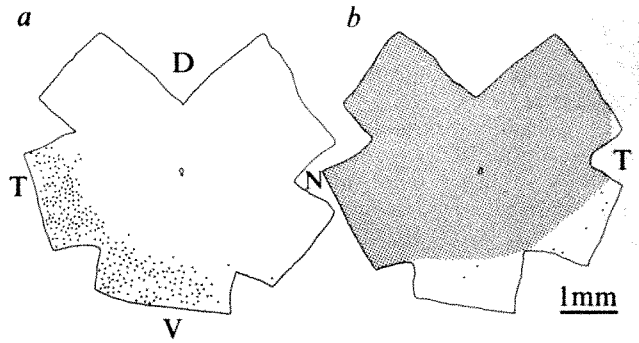


Fig. 3 Camera lucida tracings of ganglion cells labelled by anti-200K NF antibodies after optic tract section 6 days previously. Ipsilateral to the lesion (*a*) the labelled ganglion cells were regularly spaced within a crescent-shaped area in the ventral and temporal retina. The inner border of this crescent corresponds to the zero vertical meridian of the visual field[12]. Contralateral to the lesion (*b*) the bulk of the labelled cells were located in the shaded area. Their number could only be estimated (~1,000) because in more central retinal regions they were frequently obscured by heavily labelled bundles of optic axons. A crescent-shaped area corresponding to the ipsilateral projection was largely free of labelled ganglion cell bodies except for a scattered few. This region did, however, contain neurofilamentous clubs like those described in Fig. 2. V, ventral; D, dorsal; N, nasal; T, temporal.

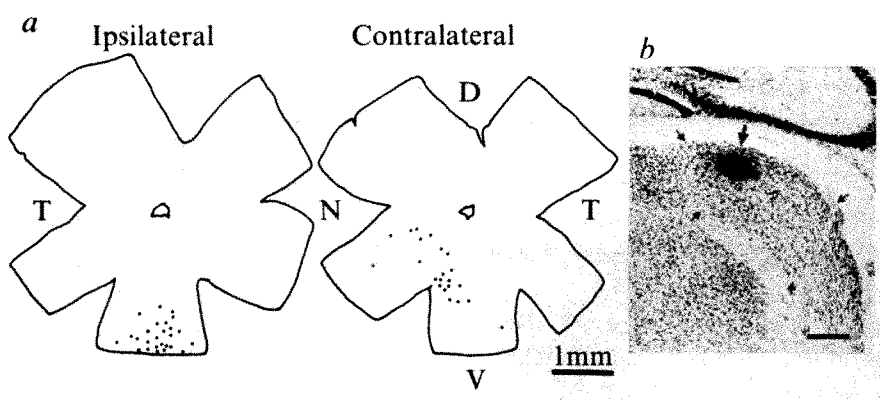


Fig. 4 Identification of ganglion cells projecting to the geniculate, using horseradish peroxidase (HRP) injections. Mice were anaesthetized with Nembutal, and visual responses were recorded from the lateral geniculate nucleus, using standard electrophysiological techniques with KCl-filled glass pipettes[26]. When a desired location within the geniculate was identified, the electrolyte was exchanged *in situ* against a solution of HRP, a tiny amount of which was then ejected electrophoretically. After 2 days the mice were perfused and the brains and retinas reacted for HRP. *a*, Camera lucida tracings of retinal whole-mounts showing positions of ganglion cells back-filled with HRP from a location in the lateral geniculate nucleus representing the upper binocular field of vision. *b*, Coronal section through the geniculate, reacted for HRP and counterstained with cresyl violet; the injection site is indicated by the large arrow, the geniculate borders by small arrows; scale bar, 200 μm. The labelled cells were from the largest population: 17.9 μm mean diameter ($n = 53$) ipsilateral to the injection and 15.6 μm ($n = 176$) contralaterally, and they were regularly spaced with comparable density and cell numbers in the two retinas. All injections into the main body of the lateral geniculate resulted in similar retinal labelling patterns, but it is possible that a population of smaller ganglion cells project to the outer shell of this nucleus. Comparable small injections into the most superficial layer of the superior colliculus label tight clusters of small ganglion cells, and deeper regions of the visual collicular layers receive a sparse and divergent input from large ganglion cells, which may be a collateral projection from the same cells that supply the lateral geniculate nucleus[26].

judged from physiological recordings. Each tectum contains a map of the entire contralateral retina, and the ipsilateral eye influence is very sparse[15,16]. The visual field representation in cortex, however, does not include the ipsilateral field (except for some infringement at the midline), and within the binocular field the influence of the ipsilateral eye is comparable with that of the contralateral eye[17]. However, although physiological observations point to a line of decussation in retinogeniculate projections, such a dividing line has never been observed anatomically in adult rodents (ref. 18 but see ref. 19). To identify the ganglion cells projecting to the geniculate, we injected horseradish peroxidase (HRP) into this nucleus. All injections confined to the main part of the geniculate labelled a population of largest ganglion cells that were spaced widely and rather regularly. When HRP was injected into the representation of the binocular field, populations of ganglion cells similar in size and spacing were labelled in both eyes (Fig. 4). In spacing, cell size and comparable bilateral distributions, the projections to the geniculate were indistinguishable from the cells that expressed the NF abnormality following optic tract lesions. This makes it likely that the line of decussation in the projections of the NF-rich ganglion cells is the anatomical substrate that accounts for the physiologically observed absence of ipsilateral field representation in visual cortex.

The time lapse to the appearance in the cell bodies of the antigenic change in response to lesions depended on the distance of the axotomy from the eye; it took about 6 days following optic tract lesions, and about 3 days after optic nerve cuts, suggesting the retrograde transmission of an injury message at ~1.5 mm per day. The antigenic change persisted for several weeks, but seemed to reverse gradually; 60 days after the lesions only a few cell bodies were still labelled, but the labelled axon segments were still extended, indicating that the cells had probably not degenerated yet but were undergoing some temporary recovery.

Here we have shown that antibodies to the heavy NF subunit which normally fail to label proximal neuronal regions, do so following lesions of the axons. The inability to label proximal cells normally may reflect the absence of the heavy NF subunit from this location, or, more likely, an affinity of the antibodies for a modified site that is slowly created as the neurofilaments are transported down the axon. The latter explanation was suggested by the Sternbergers, who showed that several anti-200K NF antibodies are directed against phosphorylated sites

not present in cell bodies[20]. Thus, one possible explanation is that the pathological changes observed here indicate atopic phosphorylation of neurofilaments in response to injury.

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Amplification of specific DNA sequences correlates with multi-drug resistance in Chinese hamster cells

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Mammalian cells selected for resistance to certain cytotoxic drugs frequently develop cross-resistance to a broad spectrum of other drugs unrelated in structure to the original selective agent[1,2]. This phenomenon constitutes a major problem in cancer chemotherapy. Multi-drug resistance arises from decreased intracellular drug accumulation[1–4], apparently due to an alteration of the plasma membrane[2,5–7]. The observation of double minute chromosomes or homogeneously staining regions in some of the multi-drug-resistant cell lines[8–12] suggests that gene amplification underlies this phenomenon. We have used the technique of DNA renaturation in agarose gels[13] to detect, compare and clone amplified DNA sequences in Adriamycin- and colchicine-resistant sublines of Chinese hamster cells[3,12]. We show that both Adriamycin- and colchicine-resistant cells contain amplified DNA fragments, some of which are amplified in both of these independently derived cell lines. Furthermore, loss of the multi-drug resistance phenotype on growth in the absence of drugs correlates with the loss of amplified DNA. These results strongly suggest that the DNA sequences which are amplified in common in multi-drug-resistant cell lines include the gene(s) responsible for a common mechanism of multi-drug resistance in these cells. We have cloned one of the commonly amplified DNA fragments and show that the degree of amplification of this fragment in the cells correlates with the degree of their drug resistance.

Table 1 Multi-drug resistance and 1.1-kb fragment amplification in different cell lines

Cell line	Relative resistance to: Adriamycin	Colcemid	Degree of 1.1-kb fragment amplification
V79	1	1	1
77A	5	7	5
LZ	3,000	436	60
LZ revertant	7		10
CHO Aux Bl	1	1	1
C5	150	280	20

Isolation and characterization of the Adriamycin-resistant sublines of V79 cells, 77A and LZ, have been described previously[12,23]. Cell lines CHO Aux B1 and C5 (ref. 3) were gifts from Dr V. Ling (University of Toronto). LZ revertant cells were obtained by growing LZ cells for 130 cumulative doublings in the absence of drug selection[12]. The degree of drug resistance of C5 cells was determined as described[23]. The degree of amplification of the 1.1-kb fragment I in different cell lines was determined from Southern blots (Fig. 2*a*) and from slot-blots (Fig. 2*b*) by cutting out and counting the bands of ^{32}P-labelled DNA.

The Chinese hamster cell lines used here are listed in Table 1. LZ, a highly Adriamycin-resistant subline of V79 cells, was obtained from a weakly resistant line 77A by multistep selection with increasing concentrations of Adriamycin[13]. The 3,000-fold degree of Adriamycin resistance in the LZ line is unstable, as LZ cells revert to an ~7-fold degree of Adriamycin resistance after prolonged growth in the absence of the drug[12]. In addition to Adriamycin, LZ cells are cross-resistant to several other drugs, including colcemid, a close analogue of colchicine[12]. C5, an independently derived subline of CHO cells selected for resistance to colchicine, is also resistant to multiple cytotoxic drugs[3]. LZ cells are relatively more resistant to Adriamycin than to colcemid (Table 1), whereas C5 cells are more resistant to colcemid than to Adriamycin. Indeed, it has been frequently observed that multi-drug-resistant cell lines are relatively more resistant to the drug used for their selection[1,2,8,10]. Such specificity suggests that while a common genetic alteration may have led to multi-drug resistance in different cases, additional genetic events may have further modified the pattern of drug resistance in different lines.

DNA from different cell lines was digested with *Bam*HI, and a portion of each DNA preparation (tracer DNA) was ^{32}P-labelled with T4 DNA polymerase[13]. Tracer DNA was mixed with an excess of unlabelled (driver) DNA and electrophoresed in an agarose gel. Following electrophoresis, DNA in the gel was subjected to two cycles of denaturation, renaturation and *in situ* digestion with S_1 nuclease to enhance selectively the signal from reiterated DNA fragments[13]. When both tracer and driver DNA derive from the same DNA preparation, this treatment produces a series of bands corresponding to the *Bam*HI restriction fragments that are reiterated at least 15–30 times per haploid mammalian genome. When tracer and driver DNA are from different DNA preparations, only those DNA fragments that are reiterated both in the tracer and in the driver are detected[13]. As the amplified chromosomal domains are known to contain varying amounts of flanking sequences that have been co-amplified together with the essential gene[14,15], identification of the commonly amplified sequences in different independently derived cell lines by the above procedure allows one to delineate the region which is likely to contain the gene of interest.

Figure 1 shows the results of experiments in which DNA was electrophoresed and then subjected to the treatment described above in either a 1% (Fig. 1*a–l*, *u–x*) or 0.7% (Fig. 1*m–t*) agarose gel. Several bands corresponding to repeated *Bam*HI fragments of hamster DNA are observed in the digest of parental V79 DNA (Fig. 1*a*, *m*, *u*). DNA from the weakly resistant line 77A (Fig. 1*b*, *n*) gives a pattern which is indistinguishable from that of V79. However, in the digest of LZ DNA (Fig. 1*c*, *o*, *v*)

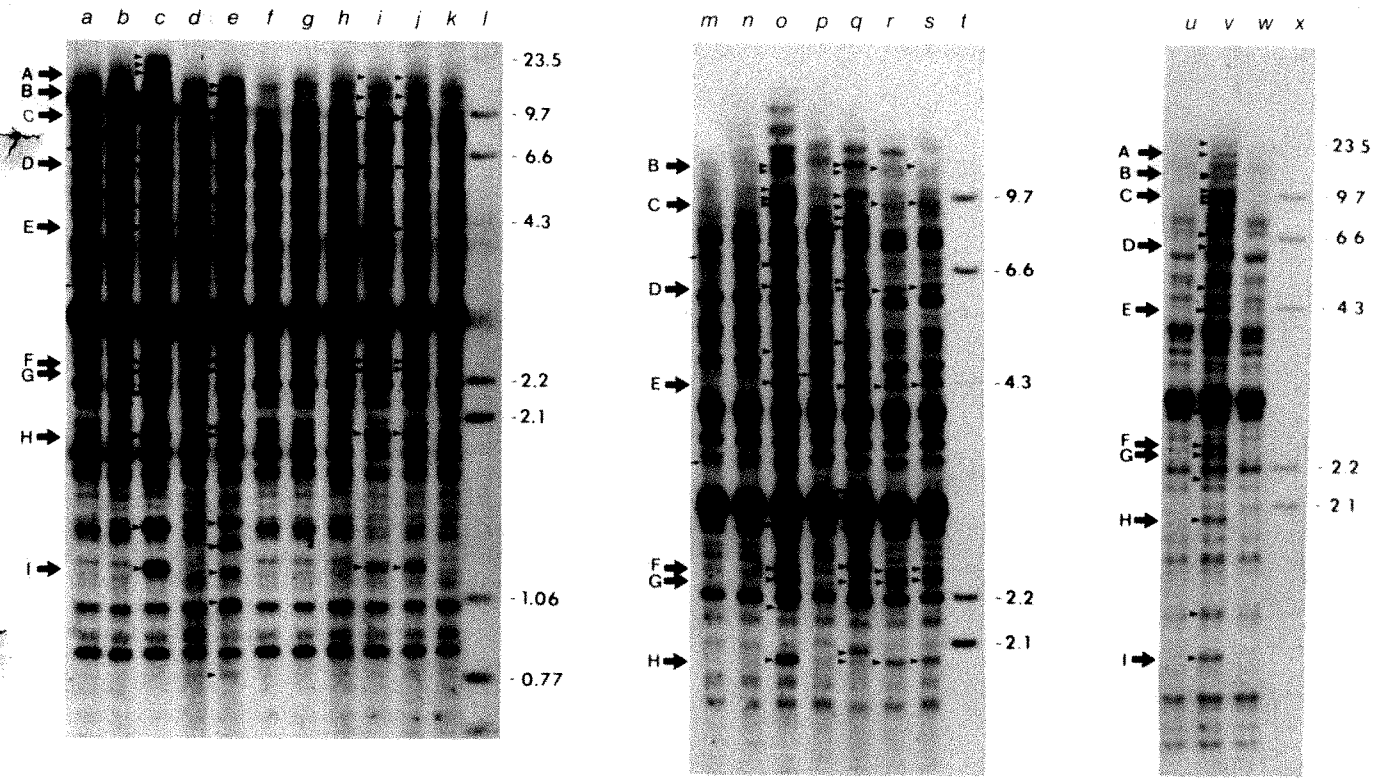


Fig. 1 Identification of amplified DNA fragments by in-gel DNA renaturation. Lanes *a–l*, *v–x*: 1% agarose gel; lanes *m–u*: 0.7% agarose. Each lane contains *Bam*HI digests of the following DNA samples: *a*, *m*, *u*, V79 (tracer and driver); *b*, *n*, 77A (tracer and driver); *c*, *o*, *v*, LZ (tracer and driver); *d*, *p*, CHO Aux Bl (tracer and driver); *e*, *q*, C5 (tracer and driver); *f*, LZ (tracer), V79 (driver); *g*, *γ*, hLZ (tracer), 77A (driver); *h*, 77A (tracer), LZ (driver); *i*, *r*, LZ (tracer), C5 (driver); *j*, *s*, C5 (tracer), LZ (driver); *k*, C5 (tracer), CHO Aux Bl (criver); *w*, LZ revertant (tracer and driver). *Hin*dIII fragments of λ phage DNA (*l*, *t*, *x*) and *Hin*cII fragments of ΦX174 RF DNA were used as size standards (*l*, *t*, *x*). Amplified DNA fragments are indicated by arrowheads. A subset of DNA fragments amplified in both LZ and C5 cells (A–I) are marked by arrows. Small arrows indicate the differences between the V79 and CHO DNA patterns (*a*, *d*, *m*, *p*). Several bands at the top of lanes *m–t* are missing because of gel breakage in this experiment.

Methods: DNAs from the different cell lines were extracted by the procedure of Blin and Stafford[24]. DNA was digested with *Bam*HI and tracer DNA was labelled with ^{32}P using T4 DNA polymerase and [α-^{32}P]dCTP (3,000 Ci mmol^{-1}) in conditions where an average of ~100 nucleotides were excised from each 3′ end in the exonuclease reaction before resynthesis[13]. DNA was separated in a 30.5 × 24 × 0.4 cm agarose gel. After electrophoresis, the gel slab was cut to 28.5 × 16.3 cm, placed in a flat-bottomed plastic box and subjected to two cycles of in-gel renaturation and S_1 nuclease digestion as described[13] with the following modifications. The composition of the hybridization buffer was 0.9 M NaCl, 0.5 mM EDTA, 50% formamide, 0.05 M Na-phosphate (*p*H 7.0). The buffer volume used for washes was 350 ml, and S_1 nuclease digestion was done in 250 ml of digestion buffer[13] containing 20,000 units of S_1 nuclease, after five 15-min washes with the digestion buffer. Each lane was loaded with either 1.36×10^7 d.p.m. in 0.35 μg (*a–k*, *m–t*) or 1.0×10^7 d.p.m. in 0.5 μg (*u–x*) of tracer DNA and 15 μg of unlabelled driver DNA.

a large number of additional bands can be detected, indicating the presence of amplified DNA in these highly drug-resistant cells. These bands were undetectable when V79 or 77A DNA was used either as a tracer or as a driver in hybridization with LZ DNA (Fig. 1*f*, *g*, *h*). The sizes of the amplified DNA fragments in the LZ DNA are ~150 kilobases (kb) in total, which should be considered the minimum estimate for the size of the amplified chromosomal domain in LZ cells (assuming that all of the amplified fragments are contiguous in the genome). In contrast, the amplified fragments were not detected in the DNA of the LZ cells that have reverted to a low degree of drug resistance after prolonged growth in the absence of Adriamycin (Fig. 1*w*). The loss of the amplified DNA fragments thus occurred concurrently with the loss of drug resistance in these cells, strongly suggesting that amplification of these DNA sequences underlies the drug resistance phenotype in LZ cells.

Comparison of the in-gel renaturation patterns produced by DNA from colchicine-resistant C5 cells (Fig. 1*e*, *q*) and from parental CHO DNA (Fig. 1*d*, *p*) reveals the presence of several amplified *Bam*HI fragments in C5 DNA; these fragments were not detected when CHO DNA was used as a driver (Fig. 1*k*). The amplified fragments in LZ and C5 DNA were compared in experiments in which LZ DNA was used as a tracer and C5 DNA as a driver (Fig. 1*i*, *r*) and vice versa (Fig. 1*j*, *s*); from the results we identified nine fragments (A–I) of total size ~55 kb that have been amplified in both LZ and C5 genomes. Amplification of the same DNA sequences in two cell lines that had been independently selected for resistance to different drugs strongly suggests a common mechanism for multi-drug resistance in these two cell lines. The gene(s) responsible for this mechanism is probably contained in these commonly amplified DNA sequences.

While the DNA fragments that are amplified in both LZ and C5 cells appear as a subset of strong bands in LZ DNA (Fig. 1*c*, *o*), the corresponding bands in C5 DNA (Fig. 1*e*, *q*) are barely detectable against the background. On the other hand, the digest of C5 DNA contains several strong bands of amplified DNA with a total size of ~70 kb, none of which is also amplified in LZ DNA. The result suggests that C5 DNA contains at least two different sets of amplified DNA fragments, which may correspond to two different amplified chromosomal domains. The domain that overlaps with the amplified domain in LZ DNA is amplified weakly in C5 cells, while the other set which is unique to C5 DNA is amplified to a greater degree. The latter sequences may carry the gene(s) specifying an additional mechanism for drug resistance in C5 cells, possibly specific for cells selected for colchicine resistance.

To address the nature of the gene(s) contained in amplified DNA, we have cloned one of the *Bam*HI fragments that have been commonly amplified in LZ and C5 DNA (the 1.1-kb fragment I in Fig. 1). LZ DNA (100 μg) was digested with *Bam*HI and separated in a 1% agarose gel. Positions of the

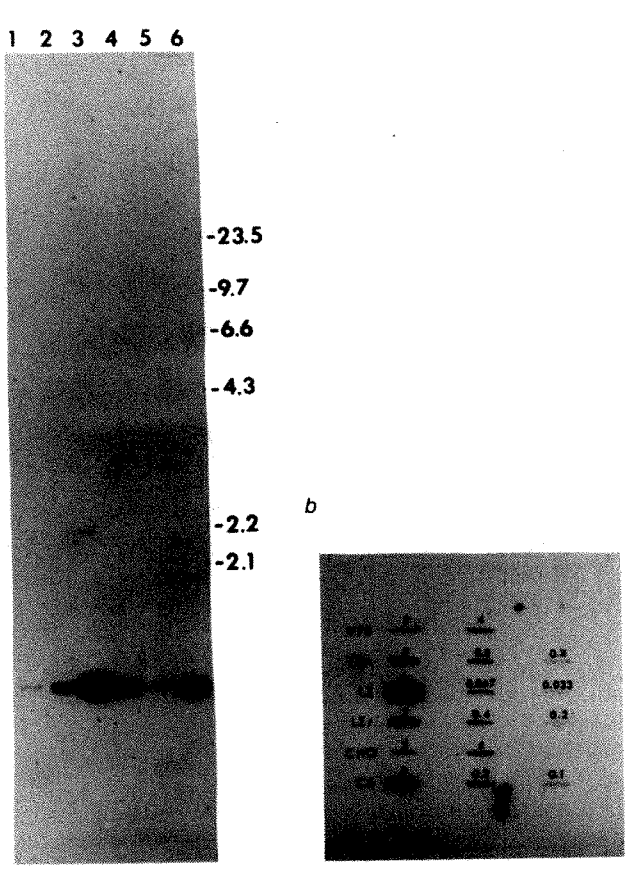


Fig. 2 Southern blot (*a*) and slot-blot (*b*) hybridization of the plasmid pDR1.1 with genomic DNA. *a*, DNA was extracted from V79, 77A, LZ, LZ revertant, CHO Aux B1 and C5 cells (lanes 1–6, respectively) and digested with *Bam*HI. Positions of the size standards are indicated. *b*, The source and amount (in μg) of DNA in each slot are indicated.

Methods: *a*, Cellular DNA (5 μg) was digested with *Bam*HI, electrophoresed in a 1% agarose gel and transferred to a nitrocellulose filter[18], then hybridized with 3×10^7 d.p.m. of pDR1.1 plasmid DNA (specific activity 2×10^8 d.p.m. μg^{-1}) that was labelled with ^{32}P by nick-translation[25]. Hybridization was done at 65 °C in 5× SSC, 2× Denhardt's, 0.1% SDS, 100 μg ml^{-1} denatured salmon sperm DNA. After hybridization the filter was washed with 0.1× SSC, 0.5% SDS at 65 °C and autoradiographed. *b*, DNA was bound to nitrocellulose filters as described[19]. The blot was hybridized with 3×10^7 d.p.m. of the pDR1.1 probe. Hybridization and washing were as described for *a*.

amplified fragments were established by in-gel renaturation[13] of the lanes containing ^{32}P-labelled V79 and LZ DNA digests separated in the same gel. A narrow strip of the gel containing fragment I was cut out and subjected to two cycles of denaturation and renaturation. S_1 nuclease was not used in this protocol so that the reannealed fragments would retain the cohesive ends generated by *Bam*HI. The mixture of single-stranded and double-stranded DNA (the latter enriched in repeated DNA fragments after renaturation) was eluted from the gel and ligated to the *Bam*HI-cleaved pBR322 plasmid. Only double-stranded DNA could be ligated at this step. The recombinant plasmids were used to transform *Escherichia coli* HB101 (ref. 16), and plasmid DNA from the resulting ampicillin-resistant/tetracycline-sensitive colonies was analysed by mini-scale DNA extraction[17] and digestion with *Bam*HI and *Hin*fI. Of 35 samples of the recombinant plasmids, seven produced identical restriction patterns, indicating that the corresponding clones contained DNA inserts that were reiterated in the genome (data not shown). DNA from one of these clones, designated pDR1.1, was used as a probe for Southern hybridization[18] with *Bam*HI-digested genomic DNA. Figure 2*a* shows that the cloned 1.1-kb fragment is amplified in all drug-resistant cell lines relative to the parental lines, and that pDR1.1 does not hybridize to any bands other than the 1.1-kb fragment and therefore does not contain interspersed DNA repeats. The degree of amplification of the 1.1-kb fragment was quantitated using both Southern blot[18] (Fig. 2*a*) and slot-blot[19] (Fig. 2*b*) hydbridization procedures. The results of these assays, summarized in Table 1, indicate an approximately linear correlation between the degree of amplification of the 1.1-kb fragment and the degree of Adriamycin resistance in the weakly resistant cell lines, while in the highly resistant lines LZ and C5, the relative increase in drug resistance strongly exceeds the relative increase in the copy number of the 1.1-kb fragment. The latter result may be due either to additional genetic changes in the highly resistant cell lines (such as the amplification of the second set of DNA fragments in C5 cells discussed above), or to changes in transcriptional regulation of the amplified gene(s) at a high degree of amplification, or to the saturable nature of a biological process regulated by the product of the amplified gene(s).

The finding that gene amplification underlies the multi-drug resistance phenotype is of particular interest as this type of drug resistance does not result from overproduction of the drug targets[3], which is the case in all the other known instances of drug resistance due to gene amplification[20,21]. The nature of the protein(s) encoded by the amplified gene(s) is unknown, although the 170,000-molecular weight membrane glycoprotein and the 19,000-molecular weight cytosol protein that are frequently overproduced in multi-drug-resistant cells[1,2,6,10,22,26,27] are likely candidates. Recently, we have isolated several phage clones containing the sequences of pDR1.1 from a genomic library of LZ DNA (P. Gros and D.E.H., unpublished). These clones are being analysed for the presence of transcribed DNA sequences and for the ability to transfer multi-drug resistance via DNA transfection. Once the specific DNA segments containing the gene(s) responsible for multi-drug resistance are identified, they will be used to isolate the corresponding protein(s). Understanding the phenomenon of multi-drug resistance in tumour cells may help in the design of treatments that would be selectively cytotoxic for the resistant cells.

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Bone marrow cells give rise to distinct cell clones within the thymus

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The thymus is the major, if not the sole site of maturation of T lymphocytes from their haematopoietic precursors[1]. During embryonic life (at a few well-defined intervals, at least in birds[2]) the thymus receives thymus-homing haematopoietic precursors that give rise to antigen-specific functional T lymphocytes[3–10]. Although the number and thymic location of distinct T-cell lineages destined to form the peripheral T-cell pool are not yet well defined, at least two independent pathways have been proposed. First, thymic subcapsular lymphoblasts divide and differentiate to give rise to small deep cortical thymic lymphocytes, medullary lymphocytes[11,12] and thymus emigrants (I.W., unpublished data) and second, the medulla contains an independent self-renewing population that contains the precursors of the peripheral T-cell pool[13]. Following irradiation the thymus may be repopulated by injected haematopoietic cells presumably related to the thymus-homing haematopoietic cells of the embryo[14,15]. Here we have reconstituted irradiated mice with limiting numbers of bone marrow cells from Thy-1 congeneic donors and have found distinct clones of cells within the thymus. The pattern of reconstitution by the precursor cells indicates that two independent thymus lineages exist: cortex plus medulla, and medulla alone.

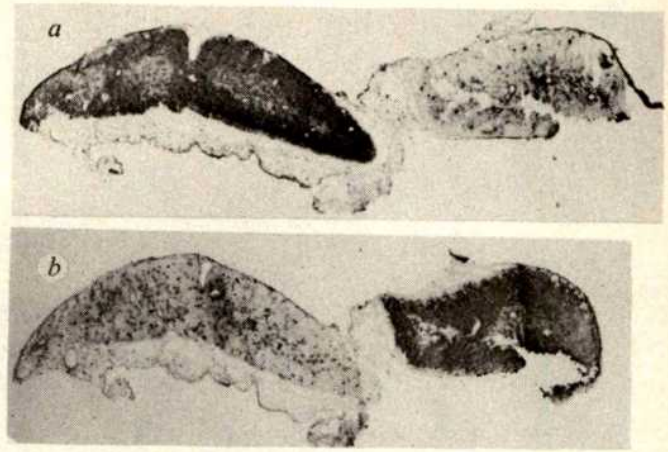


Fig. 1 Thymus from an irradiated (850 rad) C57BL/6$H\text{-}2^k$ (Thy-1.2) mouse 1 month post-reconstitution with 5×10^6 bone marrow cells (twice treated with anti-Thy-1 + complement) from a C57BL/KaThy-1.1 ($H\text{-}2^b$) donor stained with *a*, anti-Thy-1.1 and *b*, anti-Thy-1.2. Immunoperoxidase stains of frozen sections[20] were performed with monoclonal rat anti-Thy-1.2 (clone 30-H12, Becton-Dickinson) and biotin conjugated monoclonal mouse anti-Thy-1.1 (clone 19xE5) (gift of R. Nowinski) followed by rabbit anti-rat and peroxidase conjugated swine anti-rabbit (Dako) or peroxidase conjugated avidin (Vector). The left lobe is virtually entirely repopulated by donor type cells and the right lobe is virtually entirely repopulated by host type cells. Only infrequent individual cells of the opposite type are seen in each lobe.

While analysing thymic repopulation in allogeneic bone marrow chimaeras we noted one chimaera[16]—in which the allogeneic bone marrow cells differed from the host for both major histocompatibility complex (MHC) encoded antigens and Thy-1 type—that showed one lobe of the thymus entirely reconstituted with donor type Thy-1 cells, whereas the other lobe was entirely reconstituted with host-type Thy-1 cells (Fig. 1). Wallis *et al.*[17] observed that in radiation chimaeras only a few chromosomally-marked cells are needed to reconstitute an entire thymus and we were concerned that a selection process based on MHC allogenicity could have accounted for this remarkable reconstitution of two thymic lobes by two distinct stem cells. Therefore, we undertook a series of studies in which mixtures of bone marrow cells depleted of mature Thy-1^+ cells (by extensive anti-Thy-1 + complement treatment) were injected intravenously into lethally irradiated Thy-1 congeneic hosts. Mice were reconstituted with bone marrow cells varying in the ratio of donor to host Thy-1 type from 1:10 to 1:100, and thymuses were taken from hosts 2–10 weeks post-irradiation. The patterns of thymocyte repopulation were examined immunohistochemically on frozen sections using monoclonal anti-Thy-1.1 and anti-Thy-1.2 antibodies.

We have examined 80 thymus lobes among which 35 contained either extremely rare or no donor cells. The following analysis is based on the 45 lobes containing significant repopulation by donor type cells. In 5 of these lobes, discrete confluent foci of the minority population in the bone marrow mixture (the donor type) were present and ranged in size from 20 to 30 cells

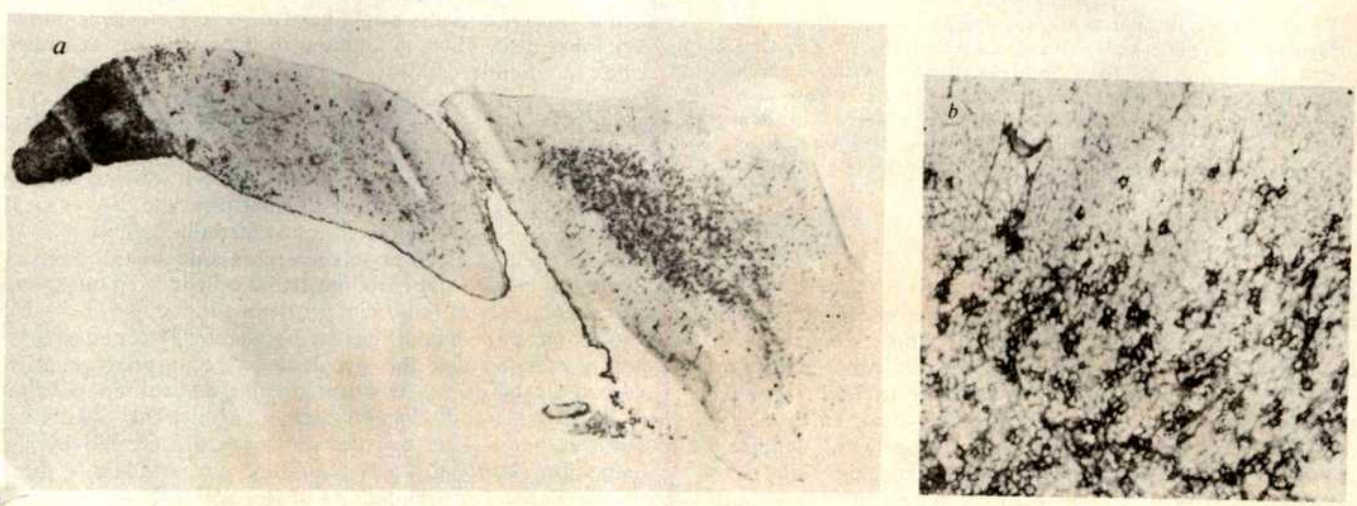


Fig. 2 Thymus of an irradiated (850 rad) C57BL/KaThy-1.1 mouse reconstituted with a mixture of anti-Thy-1 + complement treated bone marrow cells from identical and congenic (C57BL/Ka, Thy-1.2) mice, total dose 2.5×10^6 cells, Thy-1.2:Thy-1.1 (1:50), 4 weeks after reconstitution and stained here with anti-Thy-1.2 as described in Fig. 1. In this experiment and all others less than 1% of the remaining viable cells were Thy-1 positive. *a*, The left lobe contains a discrete confluent focus of donor type cells while the right lobe contains large numbers of donor type cells in the medulla with only very rare cells in the cortex. *b*, High-power view of cortex (top edge) and medulla (bottom) of right lobe, illustrating donor type (Thy-1.2) cells only in medulla.

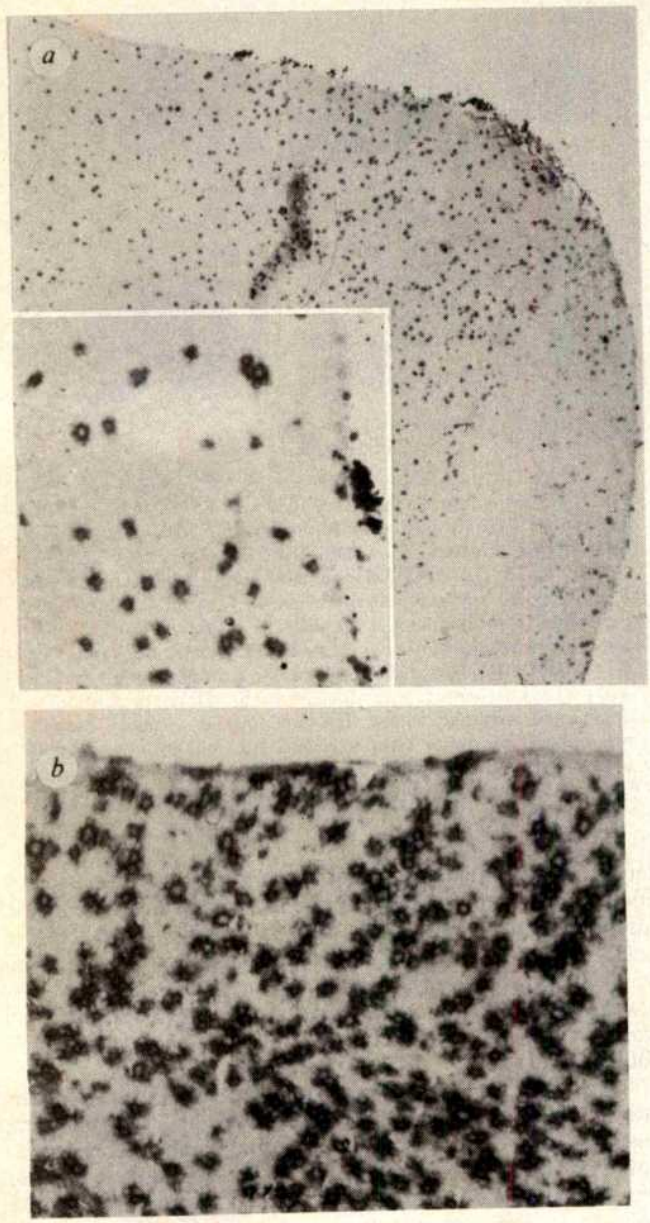


Fig. 3 Thymus of same mouse strain and reconstituted with the same total number of cells as Fig. 2, but with *a*, Thy-1.2:Thy-1.1 = 1:10, stained with anti-Thy-1.2 6 weeks after reconstitution and *b*, Thy-1.2:Thy-1.1 = 1:100, stained with anti-Thy-1.2 28 days after reconstitution. In *a* focal repopulation with scattered individual cells can be seen (cortex in lower right is negative). Inset: high-power view of individual cells. *b*, Scattered clusters of cells.

in diameter to areas of repopulation which occupied half a thymic lobe. Such foci always involved the subcapsular region, and larger foci extended to the cortico-medullary junction (Fig. 2*a*); in such cases, donor type cells were always present in the underlying medulla. In 2 of 45 thymus lobes a striking repopulation was seen: a complete reconstitution of an entire thymic lobe by the minority repopulation of donor Thy-1 type cells (1:10 ratio, 2.5×10^6 total cells transferred), with only infrequent scattered individual cells of the host Thy-1 type. This type of reconstitution is identical to that seen in the allochimaeric thymus shown in Fig. 1. The data in Figs 1 and 2 demonstrate that one or a few cells can reconstitute an entire thymic lobe.

The most frequently observed pattern of donor type cells was that of focal accumulations of scattered cells present either individually (19 of 45 thymus lobes), or as a cluster of 2–5 cells each (13 of 45 thymus lobes), generally present in both the outer and the deep cortex (Fig. 3); the medulla underlying areas of cortex containing these scattered cells also contained scattered donor type cells. We interpret these clusters and individual cells as representing the progeny of thymus homing cells of more limited proliferation potential, and perhaps of greater frequency than the fully reconstitutive thymic repopulating cells.

These various repopulation patterns could be seen as early as 13 days and as late as 10 weeks following irradiation and reconstitution. Thus we believe that we are seeing fundamental types of thymic reconstitution by incoming bone marrow cell types, and not a chance observation.

While it is possible that reconstitution occurred from multiple Thy-1 identical precursors by Thy-1 specific recruitment, preliminary experiments using bone marrow cells expressing Lyt-1 allotypic differentiation antigens also resulted in focal repopulations in three independent cases out of eight tested. We therefore conclude that these foci do not result from a Thy-1-allotype-specific congregation of cells but derive from single precursor cells.

A striking and entirely unexpected type of reconstitution occurred in 6 of the 45 thymus lobes examined: donor type thymocytes were identified only in the medulla (Fig. 2*a*, lobe on the right, and Fig. 2*b*). Multiple levels cut through these thymuses failed to demonstrate more than rare individual donor type thymocytes in the cortex. The donor type thymocytes comprised 40–60% of medullary thymocytes, and were scattered individually without clustering or localization to any specific region of the medulla. Host type thymus lymphocytes, present in abundant numbers in the cortex of these thymus lobes, made up the remainder of medullary thymic lymphocytes. While it is conceivable that medullary reconstitution could derive from recirculating mature T cells, the presence of medullary reconstitution in only one of two lobes (Fig. 2*a*) is not consistent with this pattern. All six lobes which exhibited this pattern of medullary reconstitution were from mice which had received relatively low absolute numbers of donor type bone marrow cells (1:50 ratio, 2.5×10^6 total cells transferred), and, like most thymuses, were examined 4 weeks after the transfer. Repopulation restricted to the medulla can be discerned only if there is no cortical repopulation in the same lobe by donor cells; its occurrence at higher doses of bone marrow cells may be obscured by concomitant cortical repopulations.

These findings support the proposition that the medulla may contain a separate lineage of cells which are capable of self-renewal over long intervals[13,18,19]. However, it is not possible to rule out their derivation from cortical precursors although such cortical precursors would have to be very transient and/or very infrequent. Thus, in addition to the previously described cortex to medulla pathways of development of medullary thymocytes[11,12], there could be a separate lineage of medullary cells which does not involve substantial contribution from cortical precursors in mice. Such a pathway has been reported in birds[18]. It is not clear from these experiments if the cell which is capable of repopulating the medulla is different from the cell which is capable of repopulating the cortex to medulla pathway.

The peripheral T-cell population represents the full diversity of antigen-specific and MHC-restricted cells in each functional subclass, and it is important to define the stage of thymic maturation at which such diversity is generated. We[1] and others[17] have postulated that this event could occur pre-thymically. However, experiments demonstrating oligoclonal repopulation of the thymus would seem to speak out against that possibility. However, most bone marrow chimaeras are created by the injection of 2×10^6 to 2×10^7 cells; by our calculations, these inocula would contain hundreds of clonogenic thymus-repopulation cells. Whether the full diversity of the T-cell repertoire, as well as its commitment to different functional lines, could be realized pre-thymically even with a few hundred clones is still in question.

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Accumulation of a heat shock-like protein during differentiation of human erythroid cell line K562

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The human erythroid cell line K562 provides a model system for studying erythroid differentiation and eukaryotic gene regulation. These cells express glycophorin A, spectrin and i antigen[1–4]. They accumulate embryonic and fetal haemoglobins on induction of erythroid differentiation with haemin, sodium butyrate or hydroxyurea[5,6]. In the present study, the protein composition of K562 cells during haemin-mediated induction of erythroid maturation was analysed by two-dimensional gel electrophoresis. Under conditions in which haemin did not affect cell viability and proliferation[7], a protein of ~70,000 molecular weight (MW) accumulated in the differentiated K562 cells. The accumulation appears to be due to an increase in the rate of RNA synthesis for this protein. The protein is related in sequence to a 70,000-MW heat shock protein. An antigenically related protein was also demonstrated in human bone marrow and accumulates at particular stages of human erythroid maturation.

The total protein composition and rates of protein synthesis before and after haemin induction of K562 cells were examined (Fig. 1). After 40 h of incubation with 25 μM haemin, approximately 95% of the K562 cells stained positively with benzidine. The two-dimensional electrophoretic analyses (Fig. 1) indicate that a 70,000-MW protein(s) (p70) accumulated in the haemin-induced sample (*b*), but not in the uninduced cells (*a*). Most other proteins were similar in both induced and uninduced cells. The similarity between isoelectric variants of p70 was demonstrated by two-dimensional peptide mapping analysis (data not shown). Figure 1*d*, *e* also shows that when cells were labelled with ^{35}S-methionine after induction with haemin, an enhanced labelling of the p70 occurred. We also used a cell-free system containing mRNA from haemin-treated K562 cells to show that the p70 protein represented a predominant peptide product which was not detectable among the translation products of control mRNA from untreated cells (Fig. 2). Therefore, the induction of this protein seems to be due to an accumulation of its mRNA over other cellular mRNAs.

As some heat shock stress proteins have been recently assigned a developmental role[8–10], the response of K562 cells to heat shock was investigated. Figure 1*c* shows that after exposure to 43 °C, K562 cells accumulated a series of 70,000-MW heat shock protein(s). In addition to the similarity of molecular weight and appearance in a two-dimensional gel, the heat shock p70 and the haemin-induced p70 show similarity in sequence as deduced from their similar peptide mappings described in Fig. 3 and from the digestion patterns of the two ^{35}S-labelled proteins by *Staphylococcus* protease[11].

An antigenic similarity between haemin-induced p70 and HeLa cell heat shock p70 was discovered by using antiserum to the HeLa cell proteins (given by J. Nevins). Cell extracts from control and haemin-induced K562 cells were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and the proteins electro-blotted to nitrocellulose paper[12]. Using these blots, antiserum against HeLa heat shock p70 reacted with 70,000-MW protein(s) from the K562 cells and these protein(s) were accumulated more than 10-fold in the induced cells compared with uninduced cells (Fig. 4*c*, *d*). The cross-reactive p70 in the haemin-induced K562 constituted approximately 6% of the total Coomassie-stained proteins (Fig. 4*b*). Given the immunological relatedness of haemin p70 to heat shock p70, we used a cDNA clone encoding the heat shock p70 in HeLa cells (a gift from J. Nevins) to assay the transcription rate of nascent RNA synthesis for its related protein. By using an *in vitro* nuclear transcription assay[13], it was estimated that there was a ~5-fold increase in the rate of transcription for p70 protein(s) in haemin-induced cells (data not shown). Therefore, transcriptional stimulation seems to be responsible for the accumulation of mRNA for haemin p70 in the induced K562 cells.

An antigenically cross-reactive protein was detected in extracts of human bone marrow, but not in mature erythrocytes (see Fig. *e*, *f*). Specific immunoprecipitation of a 70,000-MW protein(s) was found (Fig. 4*g*, *h*) when extracts of ^{35}S-labelled bone marrow cells were precipitated with the same antiserum against heat shock p70, but not with control serum. Furthermore, when erythrocytes and late erythroblasts in bone marrow samples were lysed by hypotonic shock[14], the 70,000-MW antigen reactive with anti-heat shock p70 was found only in the lysate and not in the pellet. This indicates that the antigen is present only in these relatively mature and lysable erythroid cells. In addition, when bone marrow cells were fractionated in a Percoll density gradient[15], the ^{35}S-labelled p70 was present in the fraction, containing predominantly erythroblasts. Therefore, human bone marrow cells, probably the late erythroblasts, have protein(s) of molecular weight 70,000 that cross-react with anti-heat shock p70.

As haemin-induced p70 accumulated in the differentiated erythroblasts of both K562 and bone marrow, its induction in K562 cells may reflect a differentiation event, rather than being a general response to stress conditions. The inducer, haemin, is a normal cellular metabolite, important for normal regulation of protein synthesis and erythroid differentiation[16,17]. The induction conditions applied did not affect cellular viability and proliferation[7], and other stress conditions such as glucose starvation did not induce accumulation of the protein in K562 cells (unpublished data). Furthermore, haemin induction of the p70 exhibits tissue specificity, as incubation of HeLa cells with haemin did not result in production of a similar protein (unpublished observation). Recently, other heat shock proteins have been found to have a developmental role. Heat shock proteins 22 and 26 of *Drosophila* are differentially transcribed during normal development[8]. The expression of four small heat shock proteins in *Drosophila* marginal disk is stimulated by ecdysterone hormone[9]. Moreover, several genes in *Drosophila* and yeast, which are related to heat shock p70, but present at different chromosomal loci, are normally expressed during development but not induced by heat shock (the heat shock–cognate genes[10]). The haemin-induced p70 protein may represent a selective activation of gene(s) 'cognate' to the heat shock genes during erythroid maturation and independent of heat shock treatment.

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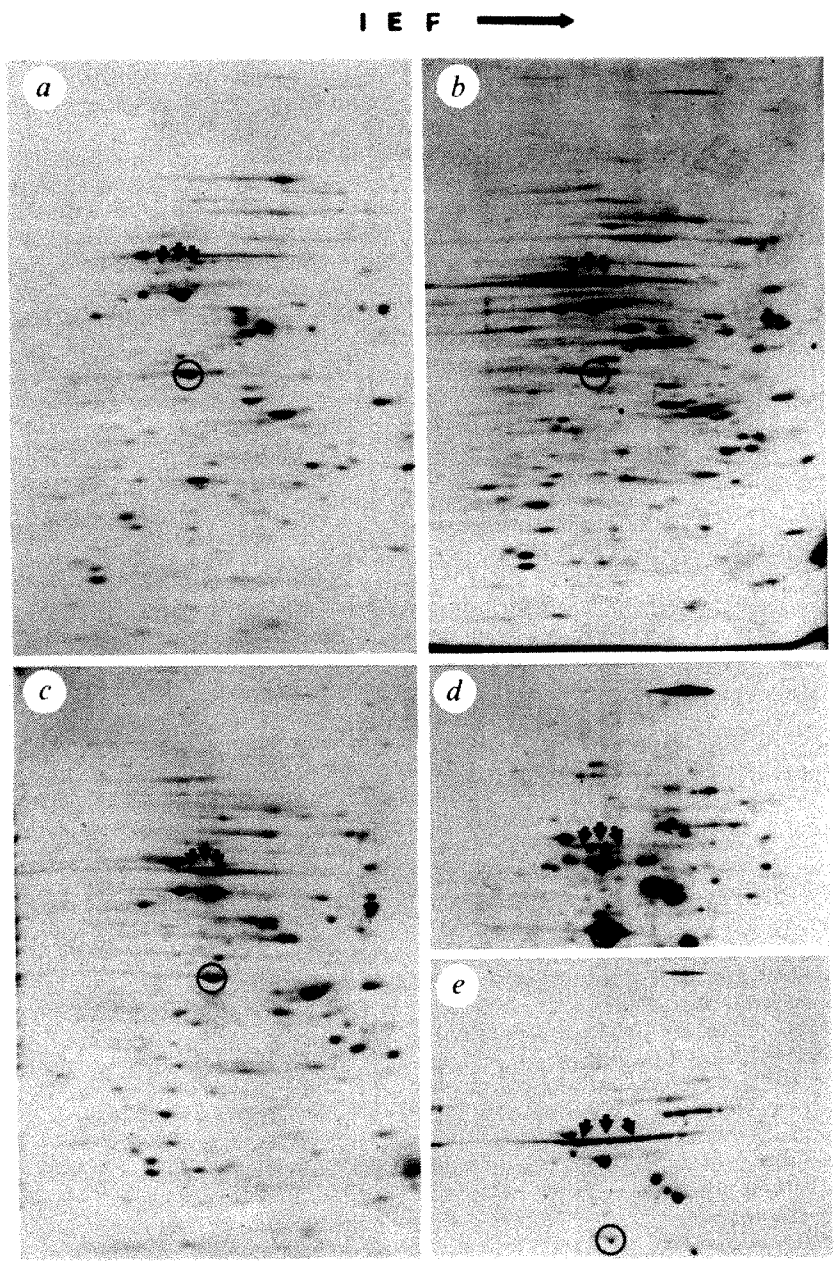


Fig. 1 Protein composition of: *a*, control; *b*, haemin- and *c*, heat shock-induced K562 cells analysed by two-dimensional gel electrophoresis. Arrows indicate the position of the 70,000-MW protein(s) and circles indicate actin. *d*, *e*, Fluorographs of the two-dimensional gels from samples of ^{35}S-labelled control and haemin-induced K562 cell, respectively. The cells were washed with Hank's balanced salt solution and labelled for 30 min in methionine-free RPMI (Gibco) medium containing 5% dialysed fetal calf serum and 50 μCi ml^{-1} ^{35}S-methionine. The fluorograph for haemin-induced cells was underexposed to show the enhanced synthesis of protein(s) indicated by arrows. Only those portions of the fluorographs with molecular weights larger than actin (indicated by circles) are shown.
Methods: K562 cells were grown in minimum essential medium containing 15% fetal calf serum and induced with 25 μM haemin for 40 h. Heat shock treatment included incubation of K562 cells at 43 °C for 1 h. The two-dimensional gel electrophoresis was done according to O'Farrell[18]. Briefly, 10^8 cells were washed with phosphate-buffered saline (PBS) and solubilized in 250 μl of lysis buffer (9.5 M urea, 2% Nonidet P-40, 4% *p*H 5–7 ampholine, 1% *p*H 3–10 ampholine and 5% 2-mercaptoethanol) and 50-μl aliquots were applied to isoelectric focusing (IEF) gel containing the same ampholine concentrations. SDS-PAGE in the second dimension was carried out on a 7.5–15% acrylamide gradient gel[19]. The gels were stained with Coomassie blue as described elsewhere[20].

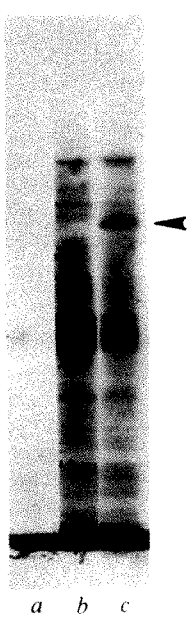


Fig. 2 *In vitro* protein synthesis directed by mRNA isolated from haemin-induced and uninduced K562 cells. RNA was isolated from uninduced K562 cells or cells induced in the presence of 25 μM haemin for 48 h. After washing with PBS, the cells were solubilized in 4 M guanidine thiocyanate and RNA prepared by pelleting through a CsCl gradient as described[21]. The poly(A)-containing RNA was isolated by oligo(dT)–cellulose chromatography, translated in a rabbit reticulocyte lysate system and analysed by electrophoresis and fluorography. The sources of mRNA used in the *in vitro* translation are as follows: *a*, no mRNA; *b*, uninduced K562 cells; *c*, haemin-induced K562 cells.

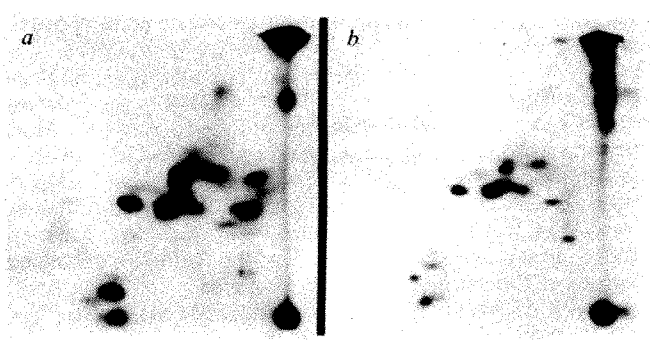


Fig. 3 Comparison of ^{125}I-labelled tryptic peptides of the haemin(*a*) and heat shock(*b*)-induced p70 of K562 cells. Gel slices were excised from the two-dimensional gel in Fig. 1*b* and *c* containing the p70 proteins and radioiodinated as described[22]. After extensive washings, the proteins were cleaved with 5 μg trypsin. Peptide analysis was performed by loading approximately 1 × 10^6 c.p.m. onto each cellulose-coated TLC plate. The samples were electrophoresed and chromatographed as described elsewhere[22]. Finally, the plates were dried and analysed by autoradiography.

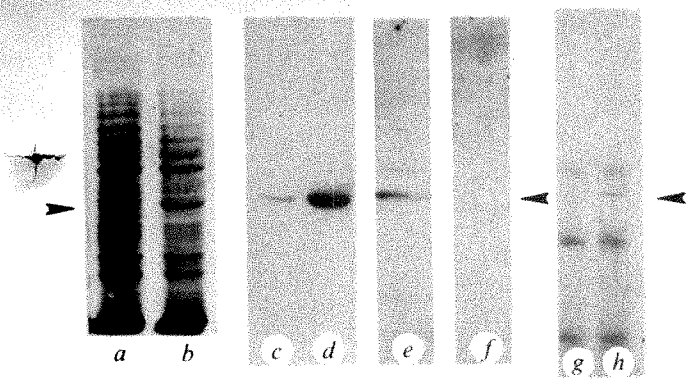


Fig. 4 Cross-reaction of a 70,000-MW protein in K562 cells and human bone marrow with antiserum against HeLa cell heat shock p70. K562 cells were induced with haemin as described in Fig. 1 legend. Equivalent aliquots of control (*a*, *c*) and induced (*b*, *d*) cells were washed extensively, solubilized by boiling in SDS-lysis buffer (62.5 mM Tris-HCl *p*H 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol and 0.001% pyronin Y), then analysed on a 7.5% acrylamide gel. Samples for *e* and *f* contained approximately 50 μg of SDS-solubilized proteins from human bone marrow and adult erythrocytes, respectively. After electrophoresis, lanes *a* and *b* were stained with Coomassie blue and lanes *c*–*f* were electroblotted to nitrocellulose paper[11] and probed sequentially with antiserum against HeLa cell heat shock p70 and ^{125}I-labelled protein A. In other experiments, equal aliquots of the same cell extracts from the ^{35}S-labelled human bone marrow cells were immunoprecipitated (*g*, with control serum; *h*, with antiserum against heat shock p70). They were then electrophoresed in a 7.5–15% gradient gel containing 1% SDS. Arrows indicate the position corresponding to the 70,000-MW protein.

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Glycoprotein C of herpes simplex virus 1 acts as a receptor for the C3b complement component on infected cells

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Receptors for the Fc portion of immunoglobulins or for the third component of complement (C3) are present on a variety of circulating and fixed tissue cells including granulocytes, monocytes, lymphocytes and glomerular epithelial cells[1–4]. Cells which lack Fc receptors may express them after infection by herpes simplex virus (HSV)-1, HSV-2, cytomegalovirus or varicella zoster virus[5–8]. We recently reported that infection by HSV-1 induces both Fc and C3 receptors on human endothelial cells[9]. Glycoprotein E of HSV-1 has been shown to function as an Fc receptor[10]. We now demonstrate that glycoprotein C (gC) of HSV-1 functions as a C3b receptor. This receptor appears following HSV-1, but not HSV-2, infection. Detection of the C3b receptor is blocked by monoclonal antibodies to glycoprotein C (gC) of HSV-1, but not by monoclonal antibodies to other HSV-1 glycoproteins. In addition, the MP mutant of HSV-1, which lacks gC, fails to express a C3b receptor. These results assign a new function of gC of HSV-1 and demonstrate potentially important differences between HSV-1 and HSV-2 glycoproteins.

Figure 1*a* shows the binding of C3b-coated erythrocytes to HSV-1 infected endothelial cells by light microscopy. Rosettes (>4 erythrocytes per endothelial cell) were found with 70% of infected endothelial cells: no rosettes formed around uninfected cells, nor around infected cells incubated with IgM- coated erythrocytes or with erythrocytes coated with complement components (C142) but not C3 (Fig. 1*b*). The specificity of the C3b-coated erythrocytes for C3b receptors was demonstrated by the ability of purified C3b fragments totally to inhibit rosetting. In contrast, purified C3 failed to block rosettes.

A second assay system was used to detect C3 receptors on infected endothelial cells (Fig. 1*c*). Complement-coated bacteria were prepared as indicator cells by incubating *Salmonella typhi* with antibody-negative fresh human serum[11]. Extensive attachment of bacteria occurred to HSV-1 infected endothelial cells. No attachment occurred to uninfected cells. Similarly no attachment occurred to infected cells when bacteria were incubated with heat-inactivated serum rather than fresh serum as the source of complement.

These results suggested that the receptor might be a viral glycoprotein. To test this, endothelial cells were infected with HSV-1 in the presence of tunicamycin to inhibit glycoprotein expression[12,13]. 3 μg ml^{-1} of tunicamycin added 1 h post-infection decreased from 70% to 5% the number of infected cells showing rosettes of C3b-coated erythrocytes at 24 h. Figure 2*a* shows the results expressed as the % binding of ^{51}Cr-labelled, C3b-coated erythrocytes.

Experiments were performed with several different HSV-1 and HSV-2 strains. Four low passage clinical isolates of HSV-1 all induced C3b receptors (Fig. 2*b*). Light microscopy revealed rosettes of C3b coated erthrocytes around 70–75% of the infected endothelial cells following infection with each HSV − 1 strain but no rosettes were found with clinical isolates of HSV-2 by either the ^{51}Cr-erythrocyte binding assay (Fig. 2*c*) or microscopic examination. All HSV-1 and HSV-2 strains induced IgG Fc receptors as measured by binding and rosetting of IgG-coated, ^{51}Cr-labelled erythrocytes (results not shown).

Glycoproteins A/B, D and E of HSV-1 have antigenic determinants in common with the equivalent glycoproteins of HSV-2 but gC of HSV-1 is predominantly type-specific[14–16]. To examine whether gC acts as the C3b receptor, we looked for expression of the receptor in cells infected with the MP mutant of HSV-1, which fails to express gC[17]. No C3b receptor activity was detected in these cells, as measured by the ^{51}Cr-erythrocyte binding assay (Fig. 2*c*) or by rosetting. In contrast, expression of Fc receptors by the MP mutant was normal, as detected both by the ^{51}Cr-binding assay and by rosetting of IgG-coated erythrocytes (results not shown).

To define further the role of gC of HSV-1 as a C3b receptor, blocking assays were performed using a series of anti-herpes antibodies as described in the legend to Fig. 3. When HSV-1 infected endothelial cells were incubated with antiserum against HSV-1 envelope antigens[15], binding of ^{51}Cr-labelled, C3b-coated erythrocytes to infected cells was markedly reduced (Fig. 3). Monoclonal antibodies to gA/B, gD and gE had no effect on binding of these erythrocytes when antibodies were tested at a 1 : 10 dilution (Fig. 3) or undiluted (results not shown). In contrast, monoclonal antibody to gC reduced markedly the binding of ^{51}Cr-labelled, C3b-coated erythrocytes. This reduction

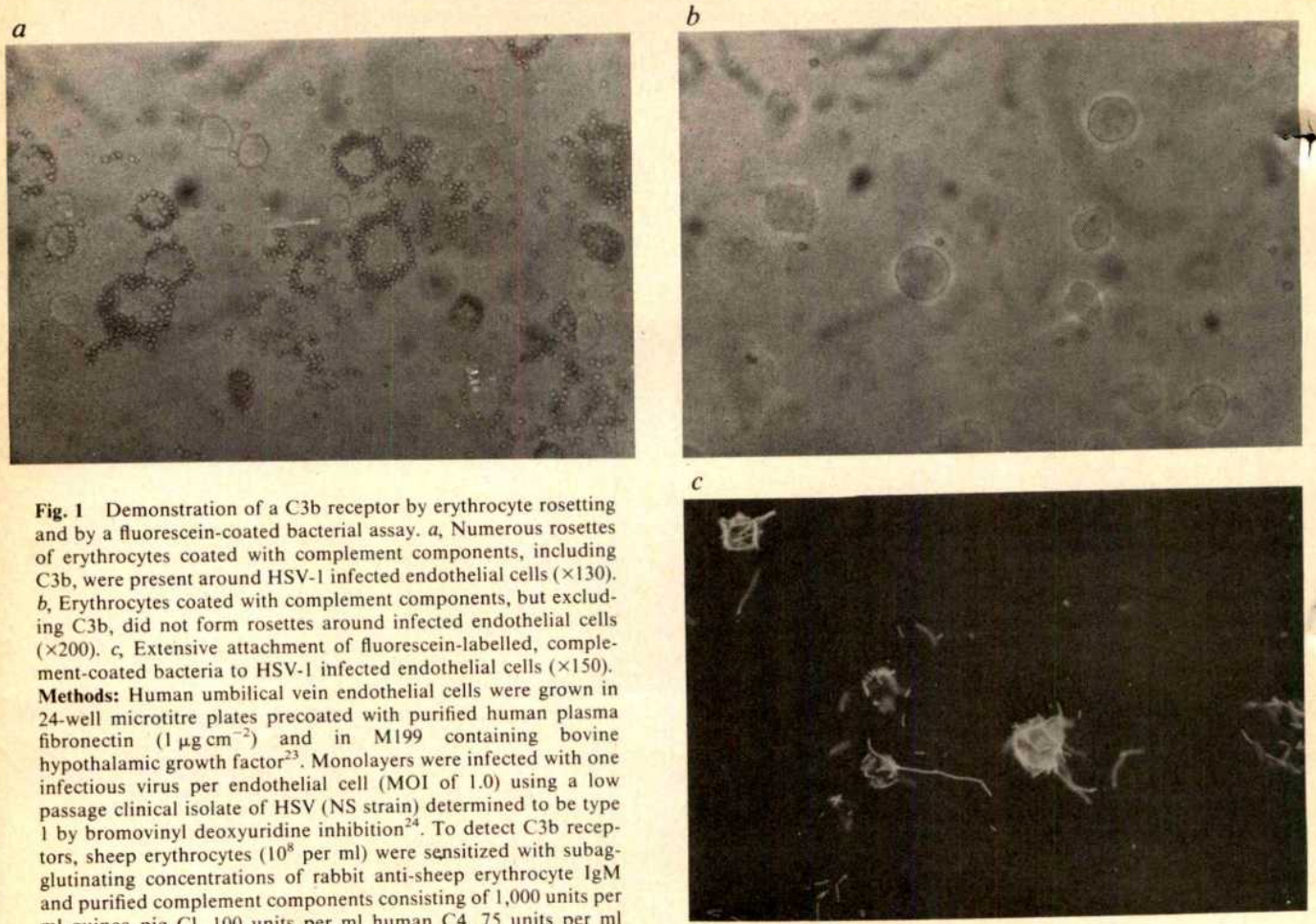


Fig. 1 Demonstration of a C3b receptor by erythrocyte rosetting and by a fluorescein-coated bacterial assay. *a*, Numerous rosettes of erythrocytes coated with complement components, including C3b, were present around HSV-1 infected endothelial cells (×130). *b*, Erythrocytes coated with complement components, but excluding C3b, did not form rosettes around infected endothelial cells (×200). *c*, Extensive attachment of fluorescein-labelled, complement-coated bacteria to HSV-1 infected endothelial cells (×150). **Methods:** Human umbilical vein endothelial cells were grown in 24-well microtitre plates precoated with purified human plasma fibronectin (1 $\mu g\,cm^{-2}$) and in M199 containing bovine hypothalamic growth factor[23]. Monolayers were infected with one infectious virus per endothelial cell (MOI of 1.0) using a low passage clinical isolate of HSV (NS strain) determined to be type 1 by bromovinyl deoxyuridine inhibition[24]. To detect C3b receptors, sheep erythrocytes (10^8 per ml) were sensitized with subagglutinating concentrations of rabbit anti-sheep erythrocyte IgM and purified complement components consisting of 1,000 units per ml guinea pig C1, 100 units per ml human C4, 75 units per ml guinea pig C2 and 100 units per ml human C3 (Cordis Labs)[9,25]. Sheep erythrocytes were also prepared as above but C3 was omitted. Sheep erythrocytes without complement components were used in some experiments and prepared as described previously[9]. Erythrocyte rosetting assays[9] were performed 24 h post-infection using a ratio of 100 erythrocytes per endothelial cell. Heat-killed *Salmonella typhi* were fluoresceinated and incubated for 15 min with human serum as a source of complement from a donor lacking antibodies to *Salmonella*.[11] 24 h after HSV-1 infection, endothelial cells were removed by scraping and were incubated for 30 min with complement-coated bacteria.

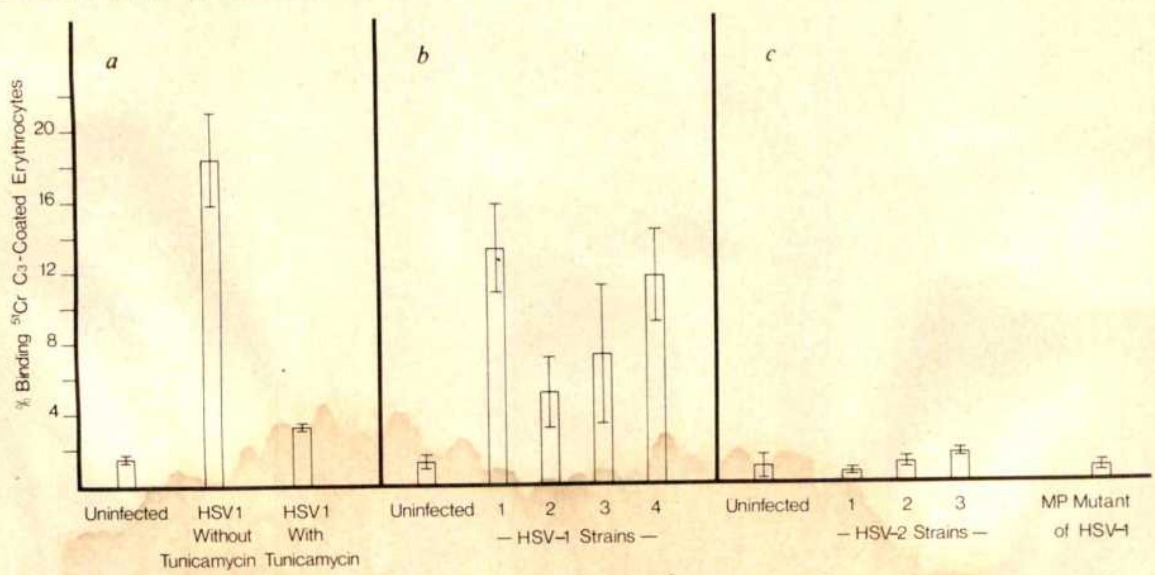


Fig. 2 Binding of coated erythrocytes to infected endothelial cells. *a*, Endothelial cells were infected with HSV 1 (NS strain) at an MOI of 1.0. One hour after infection, tunicamycin, 3 $\mu g\,ml^{-1}$, was added to some cultures and 24 h after infection sheep erythrocytes coated with IgM and C1, 2, 3, 4 were added to detect C3b receptors. *b*, 24 h after infection of endothelial cells at an MOI of 1.0 with 4 HSV-1 low passage, clinical isolates, sheep erythrocytes coated as above were added to detect the C3b receptor. *c*, Three low passage HSV-2 strains and the MP mutant of HSV-1 were used at an MOI of 1.0 to infect endothelial cells. 24 h later, sheep erythrocytes coated as above were added to detect C3b receptors. At this time cytopathic changes were present in 100% of cells and 10^6–10^7 $TCID_{50}$ infectious viruses were being produced per culture well. Erythrocytes were radiolabelled with 100 μCi ^{51}Cr (Na_2CrO_4,) per 10^9 cells and prepared as described in the legend to Fig. 1. Binding assays were performed as described previously[9] and expressed as the ratio of ^{51}Cr-labelled erythrocytes bound to the monolayer to the total ^{51}Cr-labelled erythrocytes added to the monolayer ×100%. Each bar is the mean of two experiments.

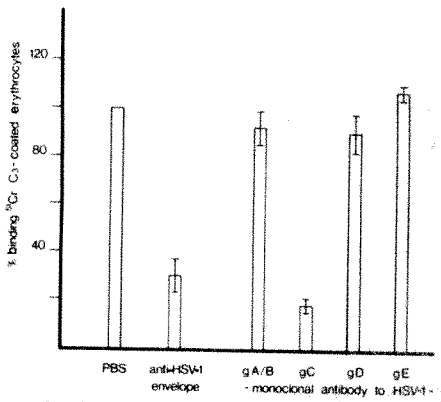


Fig. 3 Herpes antisera block the binding of C3b-coated erythrocytes to HSV-1 infected endothelial cells. Endothelial cells were infected with HSV-1 (NS strain) at an MOI 0f 1.0. 24 h later the monolayers were incubated for 30 min with 0.1 ml of either phosphate-buffered saline (PBS), antisera to HSV-1 envelope glycoproteins, or monoclonal antibodies to HSV-1 gA/B, gC, gD or gE. Unbound antiserum was removed by washing and ^{51}Cr-labelled erythrocytes, coated as described in the legend to Fig. 1, were added to detect HSV-1 induced C3b receptors. Binding of erythrocytes to infected cells in the presence of PBS was assigned a value of 100%. To calculate the effect of immune serum on blocking erythrocyte binding, the following formula was used:

$$\frac{^{51}\text{Cr-erythrocyte binding in presence of immune serum}}{^{51}\text{Cr-erythrocyte binding in presence of PBS}} \times 100\%$$

Monoclonal IgG antibodies to HSV-1, gA/B (24S), gC (5S, 17S, 19S, 27S) and gD (1S, HD1) were kindly provided by Drs B. Hamper, M. Zweig and L. Pereira. The specificity, neutralization titre, immunofluorescence activity and immunoglobulin subtypes of these monoclonal antibodies have been described elsewhere[26,27]. Additional monoclonal antibodies were prepared by the method of Holland *et al.*[14] against the low passage clinical isolate of HSV-1 (NS strain) used in these studies. Characterization of these antibodies as IgG2a anti-HSV-1 gC (1C8), gD (1D3) and gE (1E3) will be presented elsewhere (H.M.F. *et al.*, manuscript in preparation). Briefly, each of the monoclonal antibodies has neutralizing activity against HSV-1; the 1C8 antibody, however, requires complement for viral neutralization. The specificity of the monoclonal antibodies was demonstrated by radioimmunoprecipitation[15] and SDS-polyacrylamide gel analysis of cytoplasmic extracts[28] prepared from HSV-1 infected Hep-2 cells labelled with ^{35}S-methionine 5–10 h post-infection. Each monoclonal antibody immunoprecipitated proteins corresponding in size to those published for precursor and processed forms of specific HSV glycoproteins[10,28,29]. These were as follows: 1C8 anti-gC, 130,000 molecular weight (MW) and 105,000 MW; 1D3 anti-gD, 60,000 MW and 53,000 MW; 1E3 anti-gE, 80,000 MW and 68,000 MW. To block the binding of C3b-coated erythrocytes, a 1:10 dilution of ascites fluid was used. To demonstrate that binding to surface proteins occurs at this concentration, immunofluorescence studies were performed on unfixed HSV-1 infected and uninfected endothelial cells. The end-point titre was calculated as the highest dilution of antibody producing intense fluorescence on infected cells. The fluorescent antibody titres were as follows: anti-gB: 24S, 1:10; anti-gC: 17S, 1:1,000, 1C8, 1:1,000; anti-gD: HD1, 1:1,000, 1D3, 1:1,000; anti-gE: 1E3, 1:10. Each bar is the mean of two experiments except for the gC and gD monoclonal antibody experiments which are the mean of nine experiments.

occurred with each of the five anti-gC monoclonal antibodies examined at a 1:10 dilution (Fig. 3) and at a titre of 1:500 for the one anti-gC monoclonal antibody which was tested in serial dilutions (results not shown).

Anti-gC ascites (1C8) was purified by ammonium sulphate precipitation and DEAE–cellulose chromatography[18]. Purified IgG antibody (8.6 mg ml^{-1}) totally inhibited C3b–erythrocyte binding at a titre $\geq$1:1,000. None of the anti-gC monoclonal antibodies, including purified 1C8 IgG, blocked binding of IgG-coated erythrocytes to the HSV-1 Fc receptor. The anti-gE monoclonal antibody blocked binding of IgC-coated erythrocytes to Fc receptors but had no effect on binding of C3b-coated erythrocytes to the C3 receptor. All these results were confirmed by rosettes, viewed under light microscopy.

These results indicate that gC of HSV-1, either alone or in combination with endothelial cell proteins, serves as a C3b receptor. Receptors for four different cleavage products of C3 have been described on haematopoetic cells, including receptors for C3b, C3b-β1H, C3bi and C3d[4]. Whether any of these other C3 receptors are present on HSV-1 infected cells remains to be determined.

C3b receptors may serve as binding sites for complement-containing antigen–antibody complexes on certain fixed tissue cells and circulating blood cells[19,20]. If the C3b receptors induced by HSV-1 function in a similar manner, they may bind these complement-containing complexes to infected endothelial cells leading to vessel wall injury. Furthermore, C3b receptors on erythrocytes have recently been shown to inhibit activation of both the classical and alternative complement pathways[21,22]. If the viral induced C3b receptor also inhibits complement activation, the receptor may reduce the efficiency with which antibody and complement lyse infected cells but further studies are required to determine the importance of this glycoprotein in the pathogenesis of HSV-1 infection.

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Ultrabithorax mutations map to distant sites within the bithorax complex of *Drosophila*

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Chromosome breaks throughout a 70-kilobase (kb) region of the *Drosophila* bithorax complex (BX-C) result in the homoeotic *Ultrabithorax* (*Ubx*) mutations[1]. These cause the third thoracic and first abdominal segments of the *Drosophila* embryo to develop structures normally characteristic of the first and second thoracic segments[2–6]. We show here by genetic mapping that apparent point mutations with *Ubx* phenotypes are located at both ends of this 70-kb region. Mutations located at either end of the *Ubx* unit have indistinguishable phenotypes, suggesting that both classes inactivate most or all wild-type, *Ubx*$^+$, functions. This observation supports the suggestion[1] that *Ubx* mutations directly inactivate a product encoded by 5′ and 3′ exons of a long transcription unit.

Three *Ubx* mutations have previously been mapped by genetic recombination. All were found to lie at or close to the right-hand end of the *Ubx* unit defined by chromosome rearrangements[1,7,8]. When the DNA of these and other *Ubx* 'point' alleles was examined, four apparently point *Ubx* mutations were found to be associated with small insertions or deletions[1]. Three of these

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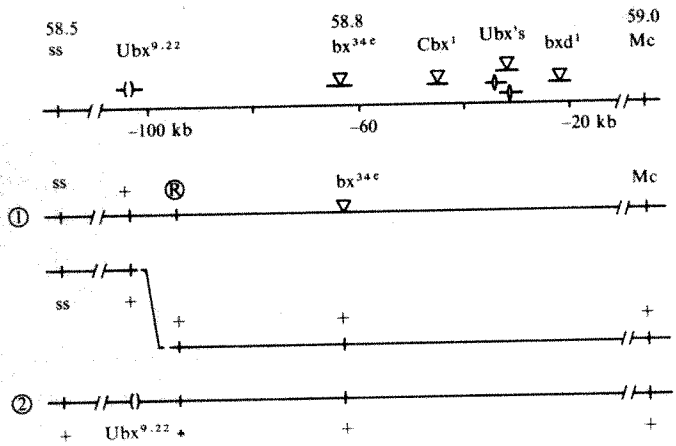


Fig. 1 Location of the $Ubx^{9.22}$ mutation. Top: Map indicating the locations of relevant loci. Numbers at the top of the diagram give the standard genetic map positions of *ss* (*spineless*), bx^{34e} and *Mc* (*Microcephalus*) on the right arm of chromosome III. The DNA scale in kilobases shows the relative locations of insertions (▽) and deletions (-( )-) in BX-C mutant chromosomes. The three *Ubx* lesions located close to −32 kb are Ubx^{1} (insertion), $Ubx^{6.28}$ and Ubx^{849} (deletions)[1]. Bottom: Segregation of the $Ubx^{9.22}$ mutant phenotype. The diagram shows the constitution of parentally derived chromosomes in non-recombinant progeny, and of one diagnostic recombinant. Plus signs indicate wild-type alleles at loci which differ between the parents. R is a restriction site polymorphism characteristic of the bx^{34e} chromosome. All wild-type recombinants lose both the bx^{34e} insertion and the $Ubx^{9.22}$ deletion, and carry the *ss* flanking marker. In the diagnostic recombinant illustrated, recombination must have occurred to the left of −95 kb, as this recombinant chromosome carried restriction sites derived from the $Ubx^{9.22}$ chromosome at the polymorphic site. From the phenotype of these recombinants in *trans* with bx^{34e} it remains possible that two separate mutations are responsible for the phenotype of the $Ubx^{9.22}$ chromosome—a *bx* mutation located to the left of bx^{34e} and a *bxd* mutation located to the right. To eliminate this possibility, bx^{+} chromosomes from each recombinant were established in stock and examined as homozygotes, and in *trans* with a range of bithorax complex mutations, including the deficiencies *Df Ubx 109* or *Df Ubx P2* (ref. 4). In no case did these combinations reveal additional BX-C mutations remaining on the recombinant chromosomes. Thus, no DNA lesion to the right of −95 kb contributes to the phenotype of the $Ubx^{9.22}$ chromosome.

lesions were located within a short (2 kb) target at the extreme right-hand end of the *Ubx* unit, within the region indicated by the genetic mapping. In the fourth case the only detectable lesion on the mutant chromosome was a 1.4-kb deletion located 70 kb from this site, at the extreme left end of the *Ubx* unit. The mapping crosses detailed below show that the *Ubx* mutation on this chromosome ($Ubx^{9.22}$) co-segregates with the observed deletion, and must be associated with DNA sequences to the left of coordinate −95 kb on the DNA map (Fig. 1), beyond the loci of *bx* (*bithorax*) and *Cbx* (*Contrabithorax*) mutations.

Table 1 gives the results of mapping crosses with $Ubx^{9.22}$, and with other previously unmapped *Ubx* alleles. Crosses with the $Ubx^{6.28}$ mutation provide appropriate controls; $Ubx^{9.22}$ and $Ubx^{6.28}$ were X-ray-induced in the same experiment, on chromosomes of common origin[9]. In both cases, the mutant chromosome reveals a deletion when compared with the parental chromosome, but whereas the deletion in $Ubx^{9.22}$ lies well to the left of the *bx* locus, that in $Ubx^{6.28}$ lies in the expected target for *Ubx* 'point' mutations, to the right of *bx* and very close to the site of the Ubx^{1} insertion[1] (Fig. 1).

$Ubx^{9.22}$ *and* $Ubx^{6.28}$ recombine with the bx^{34e} mutation at similar frequencies (Table 1). The flanking markers carried by wild-type recombinants indicate that the $Ubx^{6.28}$ mutation maps, as expected, to the right of bx^{34e}. In contrast, wild-type recombinants with $Ubx^{9.22}$ show the reverse segregation of flanking markers, confirming that the *Ubx* mutation maps to the left of bx^{34e}, and suggesting that it results from the observed deletion.

Table 1 Mapping *Ubx* alleles

Ubx allele	Cross type	Chromosomes tested	No. of BX-C^{+} recombinants	Diagnostic flanking markers on recombinant chromosome: *ss* locus	Diagnostic flanking markers on recombinant chromosome: *Mc* locus
9.22	1	10,500	7	*ss*	
9.22	2	10,400	4	*ss*	+
6.28	1	11,600	5	+	
98	2	7,600	4	+	*Mc*
154	2	13,500	4	+	*Mc*
159	2	28,000	4	+	*Mc*
195	2	22,000	0	−	−
847	2	17,000	3	+	*Mc*
849	2	13,600	3	+	*Mc*
859	2	13,000	3	+	*Mc*

Females heterozygous for each *Ubx* mutation, and for a chromosome carrying the bx^{34e} mutation in *cis* with flanking markers (see Fig. 1), were backcrossed to bx^{34e} homozygous males. Non-recombinant progeny are either bx^{34e}/bx^{34e} or bx^{34e}/Ubx. Both of these genotypes result in the partial transformation of the third thoracic segment into a second thoracic segment. Recombination between the bx^{34e} and *Ubx* mutations generates either wild-type (bx^{+} Ubx^{+}) or double mutant (bx^{34e} *Ubx*) chromosomes. The bx^{+} Ubx^{+} haplotype in *trans* with bx^{34e} gives rise to phenotypically wild-type progeny, which we have identified in these crosses. Recombinants carrying the double mutant chromosome (bx^{34e} Ubx/bx^{34e}) can be distinguished from the non-recombinant genotypes only with some difficulty; no attempt was made to identify such individuals in these crosses. The female genotype in crosses of type 1 was: *FMA3, XX/Y, SM1 Cy/+; sbd*^{2} *ss* bx^{34e}*/red sbd Ubx e.* The female genotype in type 2 crosses was *FMA3, XX/Y; +/+; In(3L)P, h sbd*^{2} *ss* bx^{34e} *Mc/sbd Ubx.* The male genotype for all crosses was: *sbd ss* bx^{34e} (ref. 14). $Ubx^{9.22}$ *and* $Ubx^{6.28}$ have been described elsewhere[9]. The remaining *Ubx* alleles were recovered by Lewis after treatment of a *su(Hw)*^{2} *sbd* chromosome with ethyl methane sulphonate; the allele numbers quoted are derived from the Lewis stock numbers. 98, 154, 159 and 195 are derived from Lewis cross 17756.98, 17756.154, 17756.159 and 17756.195; 847, 849 and 859 from cross 18136.147, 18136.149 and 18136.159 respectively. Heterozygous inversions in chromosomes I and II (series 1 crosses), or I and III left (series 2 crosses) were used to increase the frequency of recombination in the right arm of chromosome III. These enhanced recombination by a factor of ~3 (series 1 crosses, *ss*–bx^{34e} = 1.0% recombination) or 2 (series 2 crosses, *ss*–*Mc* = 1.0% recombination). Correcting for this, and for the recovery of only one recombinant class, the observed recombinants correspond to a map distance of ~0.04 from bx^{34e} to $Ubx^{9.22}$. The map distance between bx^{34e} and *Ubx* alleles located to the right (calculated on pooled data for *Ubx*s 6.28, 98, 154, 159, 849 and 859) is ~0.025 map units. The failure to observe recombinants with the Ubx^{195} allele is significant when compared with these pooled data (expected = 5.5; observed = 0; $P < 1\%$).

To define more precisely the limits within which the $Ubx^{9.22}$ allele maps, DNA was prepared from strains homozygous for the recombinant chromosomes, and restriction fragments in the region of the bithorax complex were compared with those present in the $Ubx^{9.22}$ and bx^{34e} stocks. In each of four tested cases, the recombinant chromosome lacked the restriction fragments characteristic of the *gypsy* insertion in bx^{34e} at −63.5 kb and the deleted fragments characteristic of the $Ubx^{9.22}$ chromosome at −104 kb[1] (Fig. 1). In one particularly informative case (Fig. 1), the wild-type recombinant retained restriction fragments characteristic of the $Ubx^{9.22}$ chromosome throughout the region from −30 to −95 kb, indicating that the recombination event occurred within 5 kb of the deletion present in the $Ubx^{9.22}$ chromosome. We conclude that the $Ubx^{9.22}$ chromosome carries no detectable BX-C mutation located to the right of coordinate −95 kb.

Although the $Ubx^{9.22}$ mutation maps 70 kb from the sites of the Ubx^{1} and $Ubx^{6.28}$ mutations, the phenotypes resulting from all three of these mutations are virtually indistinguishable. Each shows the same range of phenotypes when in *trans* with *bx*, *bxd* and *pbx* (*postbithorax*) mutations of the BX-C[9,10]. Homozygotes for each allele die as larvae, and those that survive to the third larval instar show partially developed anterior spiracles in the

third thoracic and first abdominal segments, which are characteristic of *Ubx* homozygotes[4].

Similar mapping crosses were performed with a series of seven other *Ubx* mutations (Table 1). One of these, Ubx^{849}, is associated with a ~110-base pair (bp) deletion in the *Ubx* 5′ target, but the remainder are candidates for true point *Ubx* mutations[1].

Ubx^{849} and five of the six point alleles map clearly to the right of the bx^{34e} mutation. Our data are consistent with the model that all of these alleles map to the same site as $Ubx^{6.28}$, approximately 0.025 map units from bx^{34e}.

Ubx^{195} does not fit this model. We observed no recombinants between this allele and bx^{34e} (see Table 1). Thus, Ubx^{195} is most likely to map at a site closer to the bx^{34e} mutation than these other alleles.

We do not know the precise location of most of the *Ubx* mutations which map to the right of the bx^{34e} locus, but in those three cases where the mutation has been associated with an identified lesion in the DNA, this lesion is located between −31.5 and −32.5 kb[1], suggesting the existence of a localized target for such mutations.

The $Ubx^{9.22}$ mutation identifies a second target for *Ubx* alleles, which must be located to the left of −95 kb. We have not proved that this *Ubx* mutation results specifically from the observed deletion, but the circumstantial evidence strongly suggests that this is so.

DNA sequences located both in the rightward *Ubx* target and in the region removed by the $Ubx^{9.22}$ deletion are present in processed transcripts of the *Ubx* unit (R. Saint and D. Hogness, unpublished results; see also refs 11–13). Taken together with these molecular data, our results support the suggestion[1] that *Ubx* mutations directly affect the expression of a product (or products) encoded by sequences which span the entire 70 kb of the *Ubx* unit.

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Xenopus oocytes can secrete bacterial β-lactamase

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Most secretory proteins are synthesized as precursor polypeptides carrying N-terminal, hydrophobic sequences which, by means of a signal recognition particle (SRP)[1,3], trigger the membrane transfer of the polypeptide and are subsequently cleaved off. The signal sequences appear to be interchangeable between prokaryotes and eukaryotes[4,5]. In bacteria, secretion only involves the crossing of a membrane[6], whereas in eukaryotes the secretory process can be separated into two distinct phases: translocation across the membrane of the rough endoplasmic reticulum and subsequent intraluminal transport by processes involving vesicle budding and fusion[7]. Since secretory proteins must be distinguished from other soluble proteins destined for various sites in the reticular system, it is conceivable that eukaryotic secretory proteins possess additional markers distinct from the signal peptide to guide the polypeptide after its transfer through the membrane. Proteins are secreted at different rates from a eukaryotic cell[8,34], suggesting a role in intracellular transport for receptors with differing affinities for some topogenic features in secretory proteins. We have tested this possibility by introducing into the lumen of eukaryotic rough endoplasmic reticulum a prokaryotic protein which, by virtue of its origin, had not been adapted to the eukaryotic secretory pathway. We reasoned that secretion of the bacterial protein would indicate that after membrane transfer no topogenic signal(s) and corresponding recognition system(s) are required. We report here that this is indeed the case.

We have chosen the β-lactamase encoded by the *Escherichia coli* plasmid pBR322 as a model for a prokaryotic secretory protein and *Xenopus* oocytes as eukaryotic test cells. β-Lactamase is the major protein secreted into the periplasm of *E. coli* cells carrying pBR322 and inactivates ampicillin, thus conferring on the bacteria resistance to the antibiotic. *Xenopus* oocytes have been shown to be a surrogate system for the study of the secretion of eukaryotic proteins (see ref. 9 for a review) or the incorporation and transport of plasma membrane proteins[10,11]. These cells, when microinjected with foreign mRNA, usually process the translation products in the same manner as the original cell and direct them to the same sites. They would therefore seem suitable for the present purpose.

β-Lactamase-specific mRNA was synthesized *in vitro* using isolated RNA polymerase from *E. coli* and plasmid DNA as template[12]. Capped mRNA was obtained by adding ^{7}mGpppA to the transcription mixture[13]. Capped mRNA appears to be stabilized against the action of 5′-exonucleases (ref. 14, and data not shown) and is also more efficient than uncapped mRNA in eukaryotic translation systems[15].

Translation of the *in vitro* synthesized RNA in the wheat germ cell-free system produced pre-β-lactamase (Fig. 1, lane 1). The *in vitro* transport of the prokaryotic polypeptide across the rough endoplasmic reticulum membrane was then tested. Addition of SRP, prepared from dog pancreas, to the translation assay abolished the synthesis of pre-β-lactamase (Fig. 1, lane 2). The translational arrest was prevented and signal peptide cleavage occurred if KCl-washed rough microsomes (K-RM) were also present during translation (Fig. 1, lane 3). These membranes are devoid of SRP[16] and inactive by themselves (Fig. 1, lane 4). Identification of the β-lactamase was based on the observation that the product processed *in vitro* by the reconstituted translocation system co-migrated with the authentic enzyme in SDS gels (compare Fig. 1, lanes 3 and 7) and that a plasmid (pKT218) lacking the nucleotide sequence coding for amino acid residues 6–181 of pre-β-lactamase[4] yielded a truncated translation product (Fig. 1, lane 5). Furthermore, cleavage with restriction enzymes within the β-lactamase gene prevented the synthesis of pre-β-lactamase after transcription–translation *in vitro*, whereas cleavage of the plasmid DNA outside the gene had no effect (data not shown).

These results are in complete agreement with those of Müller *et al.*[5] and confirm that the prokaryotic signal peptide is recognized by mammalian SRP and is cleaved by the eukaryotic signal peptidase. Removal of the signal peptide of pre-β-lactamase has also be shown previously in yeast cells[17].

As the wheat germ system possesses a capping system[15], it was important to demonstrate the synthesis of pre-β-lactamase in a reticulocyte lysate system in which endogenous capping could not occur (Fig. 1, lane 6), as a precondition for the experiments with *Xenopus* oocytes in which only pre-capped mRNAs are efficiently translated[14,18]. Synthesis was markedly

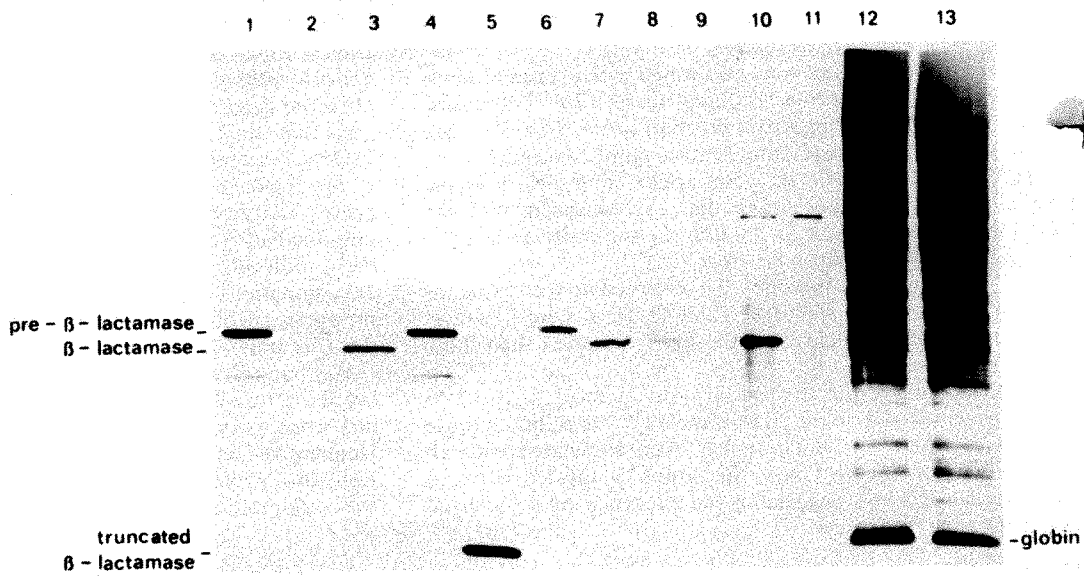


Fig. 1 Translation of *in vitro* synthesized β-lactamase mRNA in cell-free systems and in *Xenopus* oocytes. Lane 1, translation of pBR322-coded RNA in the wheat germ cell-free system. Lane 2, as lane 1 but in the presence of 5 units SRP. Lane 3, as lane 1 but in the presence of 5 units SRP and 0.5 equivalents of K-RM (for definition of units and equivalents see ref. 26). Lane 4, as lane 1 but in the presence of 0.5 equivalents of K-RM. Lane 5, translation of pKT218–coded RNA in the wheat germ system. Lane 6, translation of pBR322-coded RNA in the reticulocyte lysate system. Lane 7, ^{3}H-leucine-labelled periplasmic proteins of *E. coli* cells harbouring pBR322. Lane 8, products in the medium of four oocytes injected with pBR322-coded RNA and globin mRNA and labelled with ^{35}S-methionine (1 mCi ml^{-1}; 1,100 Ci mmol^{-1}) for 12 h (pulse). Lane 9, control to lane 8 with globin mRNA-injected oocytes. Lane 10, as lane 8 but after labelling with ^{35}S-methionine the incubation was continued for 22 h with new medium containing unlabelled methionine (10 mM) (chase). Lane 11, control to lane 10 with globin mRNA-injected oocytes. Lane 12, total products in pulse-chase labelled oocytes injected with pBR322-coded RNA and globin mRNA (the equivalent of 0.05 oocytes was applied). Lane 13, as lane 12 but oocytes only injected with globin mRNA.

Methods: β-Lactamase-specific mRNA was synthesized with isolated *E. coli* RNA polymerase and plasmid DNA as template. The assay contained in 10 μl: 0.34 units *E. coli* RNA polymerase (PL Biochemicals), 3 μg of CsCl gradient-purified pBR322 DNA[27] or pKT218 DNA[4], polyethyleneglycol 6000 (0.16 mg), RNase inhibitor from human placenta[28] (10^{-4} A_{280} units) and ^{7}mGpppA (5 nmol)[13]. Other conditions were as in ref. 5. After transcription the mixture was heated to 60 °C for 5 min. Translation of 2.5 μl transcription mixture in a 12.5-μl assay of a micrococcal nuclease-treated wheat germ system[26] or of a nuclease-treated reticulocyte lysate system[29] was performed in the presence of ^{35}S-methionine (1 mCi ml^{-1}). SRP and K-RM were isolated from dog pancreas according to ref. 16. Proteins of *E. coli* cells carrying the plasmid pBR322 were labelled with ^{3}H-leucine for 60 min and the periplasm was prepared by lysozyme–EDTA treatment[4]. For the oocyte experiments, the *in vitro* synthesized RNA was extracted with phenol–chloroform (1:1), precipitated with ethanol and dissolved in water in 1/4 of the original volume. 3 μl of this solution were mixed with 1 μl of rabbit globin mRNA (1 mg ml^{-1}) and 50 nl were injected into *Xenopus laevis* oocytes defolliculated with collagenase[10,21]. Controls received 12.5 ng globin mRNA only. The oocytes were preincubated for 2 h before labelling. Incubations were performed in Barth medium[30] in the presence of penicillin (10 μg ml^{-1}) and protease inhibitors[31]. The translation products were separated on a 10–20% (w/v) linear acrylamide gradient SDS-gel[32] and visualized by fluorography[33].

reduced in the reticulocyte lysate system with mRNA prepared in the absence of ^{7}mGpppA (data not shown).

β-Lactamase mRNA synthesized and capped *in vitro* was microinjected into oocytes and the translation products were labelled with ^{35}S-methionine. Globin mRNA was co-injected as an internal control. Globin was clearly seen among the products inside the oocyte, whereas the endogenous background did not allow a definite identification of a β-lactamase band (Fig. 1, lanes 12, 13). In the medium of oocytes incubated for 12 h with ^{35}S-methionine, a major band was seen at the position of β-lactamase (compare Fig. 1, lanes 7 and 8) which was absent from the medium of control oocytes (lane 9). The levels of β-lactamase in the medium increased after the chase period (lanes 10, 11), while globin was undetectable (lanes 8–11), excluding the possibility of nonspecific leakage from the oocytes.

Independent proof for the secretion of β-lactamase from *Xenopus* oocytes was obtained by measurement of the enzymatic activity in the medium. Nitrocefin was used as a sensitive chromogenic substrate of β-lactamase[19] (Fig. 2). The medium of oocytes injected with plasmid-coded mRNA contained significant activity whereas globin mRNA-injected controls were negative. Again, in accordance with the labelling experiments, more β-lactamase was found after the chase period than before it.

Rough estimates of the number of enzyme molecules secreted by the oocytes could be obtained from both types of experiments. Using the radioactivity in the β-lactamase band and the methionine content of oocytes[20], it was calculated that 3×10^{10} molecules per oocyte were synthesized and secreted. Using enzymatic activity it was estimated that 10^{10} molecules of β-lactamase were secreted by one oocyte in 24 h (the specific activity of pure β-lactamase was assumed to be 200 units per mg[17]). These values compare favourably with estimates of the amount of eukaryotic secretory proteins synthesized in and exported from oocytes[10,21]. Also, the time periods between synthesis of the polypeptides and their appearance in the medium

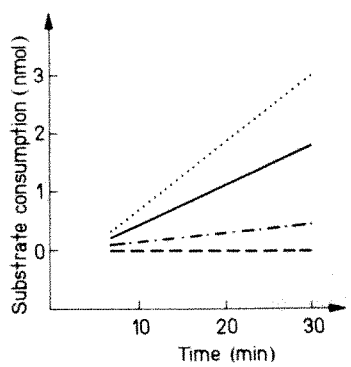


Fig. 2 Detection of β-lactamase activity in the medium of oocytes injected with plasmid-coded mRNA. The β-lactamase activity was measured with nitrocefin as a chromogenic substrate (0.2 mg ml^{-1} in 50 mM phosphate buffer, *p*H 7.0) by the increase of the absorbance at 482 nm. The medium of two oocytes injected with pBR322-coded RNA and globin mRNA was tested after 12 h pulse-incubation (—·—) or after an additional 22 h of chase (——). A test with chase medium obtained from four oocytes (·····) showed nearly double the rate. Control oocytes received globin mRNA (— — —).

are similar (see ref. 22). Thus, the bacterial secretory protein may be handled in the eukaryotic cell with similar efficiency to a genuine eukaryotic protein.

Our results indicate that a signal peptide is sufficient to trigger secretion, even in a complex eukaryotic cell, and that no further topogenic information and corresponding recognition systems are needed after traversal of the membrane. Conversely, one may assume that non-secretory, soluble proteins, located in the lumen of the reticular system of a eukaryotic cell, need some device to prevent their secretion. For lysosomal enzymes of fibroblasts, for example, this mechanism appears to be the mannose 6-phosphate receptor pathway (for review see ref. 23).

Our results do not imply that every protein without a sorting signal will be secreted once it has passed the rough endoplasmic reticulum membrane. Poor solubility or special affinity to membranes can interfere with intraluminal transport (for examples see refs 24, 25).

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Sexual preference of apparent gene conversion events in MHC genes of mice

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Polymorphisms exist at many genetic loci. At some loci, however, polymorphism is so high that tens and even hundreds of different alleles coexist in the population. Two such highly polymorphic systems are the immunoglobulin genes and the vertebrate major histocompatibility loci. The origin and maintenance of highly polymorphic loci remain open to debate but it seems likely that special mechanisms contribute to their variability and that their polymorphism serves important biological roles. The high degree of polymorphism at the *H-2* class I major histocompatibility locus of the mouse has been documented by both tissue transplantation and serological methods[1]. More recently, molecular cloning and DNA sequencing of some of the class I genes has shown that most of the sequence variability is concentrated in the first two domains and is often found in clustered regions within them[2–4]. In addition, several groups have suggested that gene conversion events among the many class I genes may contribute to *H-2* polymorphism[5,6]; such events would have to occur during meiosis to produce heritable alterations. The strongest evidence for gene conversion comes from sequence analysis of mutant class I *H-2* alleles where concerted changes at adjoining sites in DNA imply gene conversion by distant but closely related loci[5,6]. We report here an analysis of these mutants indicating that the chromosomes containing loci that have experienced gene conversion originated from females. These data suggest a striking preference for mammalian meiotic gene conversion events during female rather than male gametogenesis.

The existing *H-2* mutants were originally identified by circular skin-grafting experiments among sibling mice[7,8]. This system of mutants is notable because: (1) the rate of observed mutation is very high, about 1 in 2,500; (2) individual mutational events result in multiple amino acid substitutions; (3) independent mutants have identical, multiple changes; and (4) in most cases, the newly introduced amino acid sequences are found in the same configuration in one of the other genes of the major histocompatibility complex (MHC). Two independent groups have recently shown unequivocally that in the case of the *bm1* mutant, a gene conversion-like event resulted in the substitution of a piece of DNA in the middle of the gene, producing the K^{bm1} gene[5,6].

While considering possible mechanisms for this high rate of gene conversion we were struck, as others had been previously[9], by the apparent allele specificity of the conversion process. In the initial experiments, where skin grafts among (C57BL/6 × BALB/c)F_1 mice were performed, mutation was observed at the K^b locus to the almost total exclusion of alternative available loci (K^d, D^b, D^d and L^d). The results suggested that perhaps K^b is especially prone to alteration or possibly that changes in K^b were more easily recognized than others using a skin graft assay. The K^b gene, however, has no obvious uniqueness in its sequence and, since the initial experiments, mutations at other loci have been found. While puzzling over the existing data, a previously unrecognized feature became apparent. In the initial experiments, the F_1 mice all originated from mating C57BL/6 females with BALB/c males[7,9–11]. Thus, the K^b allele was always contributed through the egg rather than the sperm. A review of all available data in which the origin of a mutated allele could be traced because the investigators used F_1 mice, showed that in every case, the mutation arose in a chromosome contributed by the egg (Table 1)[7,8]. For this compilation, only mutants of the spontaneous gain/loss type were included because these are the ones most likely to involve a gene conversion-like event. (A gain/loss type mutant is one in which both the mutant and the parental type reciprocally reject skin grafts consistent with the simultaneous loss of one transplantation antigen and the acquisition of another.) There were 16 cases found, 9 of which involved K^b, 3 of which involved other *b* haplotype alleles and 4 of which were in maternal chromosomes from other haplotypes (Table 1). In one case, mutation in K^d was found in a strain where the *d* haplotype was maternal and the *b* haplotype paternal.

Gene conversion apparently occurred exclusively on maternal chromosomes in all cases examined. A maternal K^b allele was involved in many of these cases and if it had special properties then it would skew the data for reasons unrelated to the sex of the chromosome donor. Without the K^b events, however, all of seven cases were maternal, which would occur by chance with

Table 1 Gain/loss *H-2* mutants

Mutants	Affected locus	Maternal *H-2* haplotype	Paternal *H-2* haplotype	F_1 cross in which mutation arose
bm1	K^b	*b*	*d*	C57BL/6By × BALB/cBy
bm2	K^b	*b*	*d*	C57BL/6By × BALB/cBy
bm4	K^b	*b*	*d*	C57BL/6By × BALB/cBy
bm7	K^b	*b*	*d*	C57BL/6By × BALB/cBy
bm9	K^b	*b*	*d*	C57BL/6By × BALB/cBy
bm10	K^b	*b*	*d*	C57BL/6By × BALB/cBy
bm11	K^b	*b*	*d*	C57BL/6By × BALB/cBy
bm12	A^b_β	*b*	*d*	C57BL/6By × BALB/cBy
bm13	D^b	*b*	*d*	C57BL/6By × BALB/cBy
bm14	D^b	*b*	*d*	C57BL/6By × BALB/cBy
fm1	K^f	*f*	*a*	A.CA/SnK1/Eg × A./Sn
fm2	D^f	*f*	*r*	B10.M × B10.RIII(71NS)
KH162A	K^d–IA^d	*d*	*b*	BALB/cKh × C57BL/6Kh
KH170	K^b	*b*	*d*	C57BL/6Kh × BALB/cKh
KH171	K^b	*b*	*d*	C57BL/6Kh × BALB/cKH
dm5	K^d	*d*	*b*	BALB/cKh × C57BL/6Kh

The origins of the mutants, together with the affected loci, are shown. For completeness, the table includes mutant *bm12*, which has a mutation in the A_β gene, and KH162 which has not been further characterized other than to be in the K^d–IA^d region. For details of the various origins of these mice see refs 7, 11.

a <1% probability. Although the rates of occurrence of gain/loss type mutants vary considerably, the events at K^b do not seem to be more frequent compared with other events in other haplotypes[12]; rather, K^b events are more frequent than D^b events. We conclude, therefore, that gene conversion events (if all MHC mutants have such an origin) are much more frequent in oogenesis than in spermatogenesis and possibly never occur during male gametogenesis. Although there are no available data for analysing any other gene system, it is reasonable to expect that in other multi-gene families, like immunoglobulin variable-region genes, the same specificity of gene conversion events might pertain.

Although gene conversion events in lower eukaryotic organisms are often associated with recombination of flanking markers[13], the localized gene conversion events at MHC and immunoglobulin genes are not known to have such an association. It may be relevant, however, that the intra-*H-2* recombination rate is 0.32% in females and 0.19% in males[12]. The excess rates in females are possibly associated with gene conversion events.

Although our analysis indicates preferential if not exclusive gene conversion in maternal gametogenesis, the preference of *H-2K* conversion over *H-2D* conversion still requires explanation[7]. Perhaps there is a polarization of events along the chromosome or some specificity associated with K^b structure.

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Nonspecific integration of the HTLV provirus genome into adult T-cell leukaemia cells

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Human T-cell leukaemia virus (HTLV)[1–4], previously also reported as ATLV[5], is a recently identified retrovirus which is closely associated with adult T-cell leukaemia (ATL) endemic in south-western Japan[6,7] and the Caribbean[8]. Determination of the total nucleotide sequence of the HTLV genome has revealed no typical *onc* gene acquired from the cellular sequence[9,10]. Screening of the HTLV provirus genome in tumour cells[4,11,12] has shown that in all cases of ATL examined, the primary tumour cells contained the provirus genome and were monoclonal with respect to the integration site of the provirus[13]. These findings suggest that ATL leukaemogenesis may be due to insertional mutagenesis in which the provirus genome is integrated into a specific locus on the chromosomal DNA and then activates an adjacent cellular *onc* gene, a mechanism already demonstrated in avian lymphoma[14,15] and erythroblastosis[16] induced by avian leukosis viruses. A common site of HTLV provirus integration in leukaemic cells among some ATL patients was reported by Hahn *et al.*[17] but subsequently retracted[18]. However, this retraction does not imply the random integration of the proviruses. Independently, we have been testing this insertional mutagenesis model in ATL and report here that the provirus did not have a common locus of integration in 35 ATL patients and did not integrate on the same chromosome in 2 ATL patients.

As previously reported, the leukaemic cells of most ATL patients contain one or two copies of HTLV provirus[4,11], but the size of the *Eco*RI-digested DNA fragment containing the provirus and cellular flanking sequences differs between individuals, suggesting that the integration sites of the HTLV provirus in these patients are not identical. However, even if the actual chromosomal integration sites are different, it is still possible that proviruses integrated into one or a few common regions could explain the activation of a unique cellular gene. The strategy used was to examine the rearrangements of cellular DNA sequences by provirus integration. By using specified probes, we could detect the integration of provirus into any site within specified DNA fragments. We chose the DNA clone λATK-1 (ref. 10), which had been isolated from fresh leukaemic cells of an ATL patient (KKT). As these cells had only one copy of the provirus genome[10], the provirus must be involved in initiating or promoting leukaemic cell growth. The cellular flanking sequence of this provirus could therefore be used as a chromosomal site-specific probe to test the significance of provirus integration into the tumour cell DNA.

The unique cellular sequence flanking the 3′ long terminal repeat (LTR) was subcloned into pBR322 and used as probe *a* (Fig. 1*a*) for screening a human gene library to isolate clones covering wider regions around the provirus integration site. Isolation of three independent clones (GL1, GL2 and GL3) and construction of their restriction maps resulted in three *Sst*I fragments (A, B and C, Fig. 1*A*) covering a region of about 28 kilobases (kb) around the provirus integration site in leukaemic cell DNA of patient KKT. We subcloned the unique sequences *a*, *b* and *c* from fragments A, B and C, respectively, and used them as probes to detect DNA rearrangements in *Sst*I digests of leukaemic cell DNA from 35 other ATL patients. As *Sst*I does not cut the viral sequences, the integrated fragments with the HTLV provirus sequence should have higher molecular weights than the original fragments A, B and C. Figure 1*B* shows some results of screening using probe *a*. In addition to the normal cellular fragments, a rearranged larger fragment was detected in the control DNA of patient KKT from which the original clone was isolated, but no such rearranged fragments were detected in the DNA of the other ATL patients. These results were confirmed by rehybridization of the same filters using viral probes that detected co-migration of the only provirus sequence with the rearranged fragment of patient KKT (Fig. 1*c*). No DNA rearrangements were detected with probes *b* and *c* (data not shown). This absence of a common region for provirus integration in leukaemic cells suggests that it is unlikely that ATL is caused by simple insertional mutagenesis.

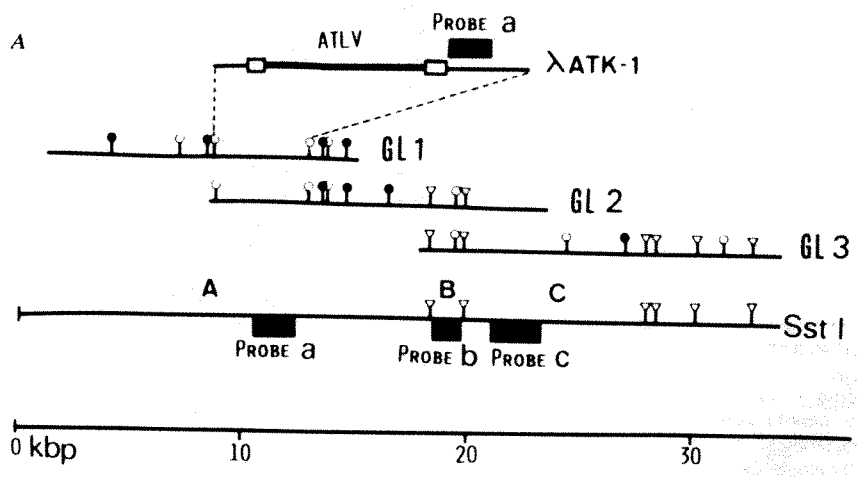
This conclusion is based on analysis of a single locus of a provirus integration in only one case of ATL, which could be an exceptional case. To exclude this possibility, we isolated another clone, λATY, from fresh leukaemic cells of another ATL patient (MYK). Blotting analysis showed that these leukaemic cells also contained only one copy of the provirus genome. The unique cellular flanking sequence in patient MYK was subcloned and used as a probe in the same way as described above. This probe (probe *d*) detected an 8-kb fragment in the *Hin*dIII digest of normal cellular DNA and a 10.5-kb rearranged fragment in the digest of DNA from patient MYK (data not shown). Again, probe *d* did not detect any rearranged fragment

Table 1 Distribution of probes *a* and *d* in somatic cell hybrids

Cell hybrids	Probes *a* (KKT)	Probes *d* (MYK)	Chromosomes determined by enzyme markers 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X*
ICL-15	−	+	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	+	−	−	+	+	−	−
WIL-14	+	+	−	−	+	−	−	−	+	+	−	+	−	−	−	+	+	−	+	−	−	−	−	−	+
WIL-6	+	+	−	+	−	+	+	+	+	+	−	+	+	−	−	+	−	−	+	−	+	+	+	−	+
WIL-5	−	+	−	−	−	+	−	−	−	+	−	+	−	−	−	−	−	−	+	+	−	−	+	−	+
WIL-2	−	+	−	−	−	−	−	−	−	+	+	−	−	+	+	−	+	−	+	−	+	−	+	−	+
WIL-1	−	+	+	+	−	−	−	−	−	+	+	+	−	+	+	+	−	−	+	+	−	−	+	−	+
ATR-15	$+^{w}$	−	+	+	+	+	+	−	+	+	−	+	−	+	+	+	+	−	−	+	+	+	+	+	+
ATR-13	+	$+^{w}$	+	+	+	+	+	+	+	+	−	+	−	+	−	+	+	+	+	+	+	−	+	−	+
ATR-11	+	−	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	−	+	+	−	+	+	+
NSL-9	−	+	−	−	−	−	+	−	−	+	−	+	−	+	+	+	+	+	+	+	−	+	+	+	−
TSL-2	−	+	−	+	+	−	+	+	−	−	−	+	−	+	−	−	−	−	+	+	−	+	+	+	−
XER-7	+	−	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	−	−	+	+	−	−	−	+
EXR-5 CSAz	$+^{w}$	−	+	+	+	−	+	+	+	+	+	+	−	+	−	+	−	−	−	+	+	−	+	+	−
% Discordancy for *a*			23	23	15	23	23	31	0	38	54	38	38	54	54	15	31	46	77	46	15	62	62	38	31
% Discordancy for *d*			85	69	77	69	69	62	77	38	69	38	69	54	69	62	62	62	0	62	77	46	31	85	46

Probe *a* (KKT), A in Fig. 3; probe *d* (MYK), B in Fig. 3. % Discordancy indicates the frequency that probes do not co-segregate with a specific human chromosome. Human chromosomes were determined by analysing each cell hybrid for enzyme markers encoded on each human chromosome as described previously[21]. $+^{w}$, weak band.

* Chromosome Y is not involved as human female parental cells were used.



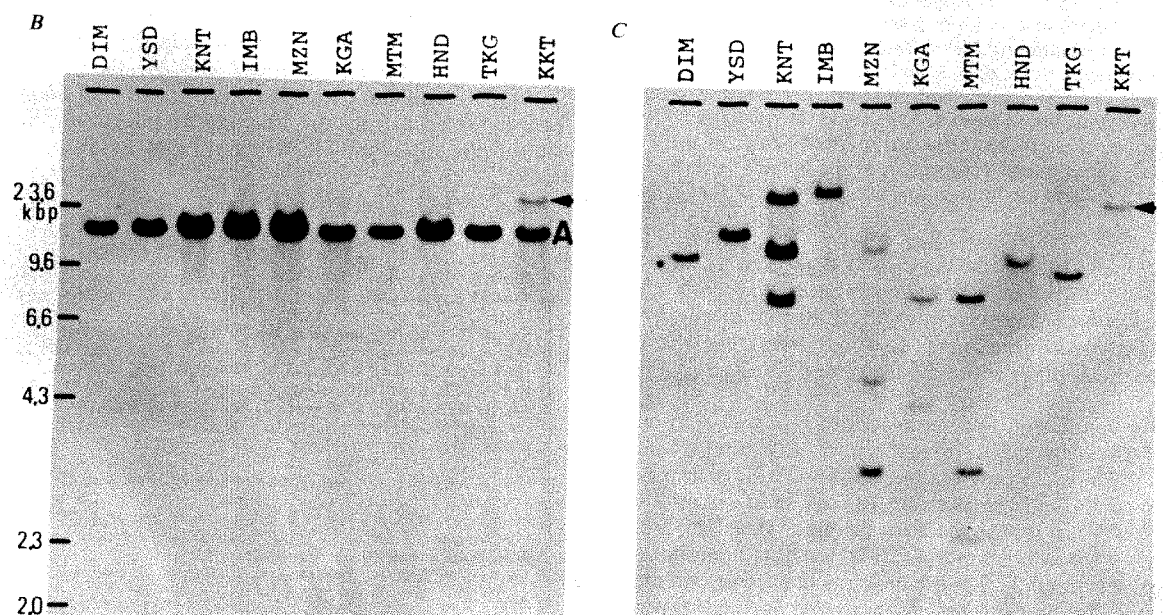


Fig. 1 Analysis of provirus integration sites in leukaemic cells from ATL patients using probes of cellular flanking sequences. *A*, Clones GL1, GL2 and GL3 of cellular DNA adjacent to the provirus genome integrated into leukaemia cells of a patient (KKT) were isolated from a human gene library using probe *a* and then probe *c*, and the restriction map was constructed. Probes *a*, *b* and *c* were separately subcloned into pBR322. ♀, *Eco*RI; ♦, *Hin*dIII; ♈, *Sst*I. *B*, DNA samples from fresh leukaemic cells of independent ATL patients were digested with *Sst*I, fractionated in 0.8% agarose gel and transferred to a nitrocellulose filter. The filter was hybridized with ^{32}P-labelled probe *a*. Lane KKT is DNA from which the original provirus clone λATK-1 was isolated, and other lanes are DNAs from other ATL patients. A indicates normal fragments detected by probe *a*. The fragment rearranged by provirus integration is indicated by an arrow. *C*, The filter used in *B* was washed in boiling water for 10 min and used for a second hybridization with ^{32}P-labelled HTLV representative probe.

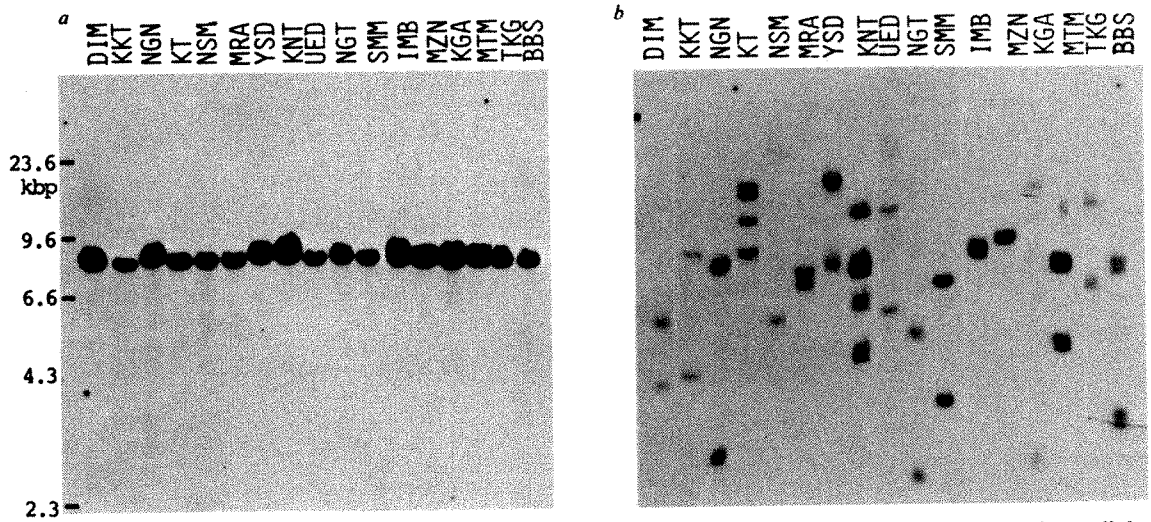


Fig. 2 Analysis of provirus integration sites in DNA of leukaemic cells from ATL patients with a second independent cellular flanking sequence. *a*, The filter was hybridized with ^{32}P-labelled probe *d*. *b*, The same filter was hybridized with ^{32}P-labelled HTLV as representative probe. Molecular clone λATY was obtained from an *Sst*I digest of DNA from another ATL patient (MYK) according to the methods reported previously[9]. The unique cellular sequence adjacent to 3′ of the provirus was subcloned from the *Hpa*II digest of λATY into pBR322 and used as probe *d*. DNA samples from ATL patients were digested with *Hin*dIII and analysed as in Fig. 1.

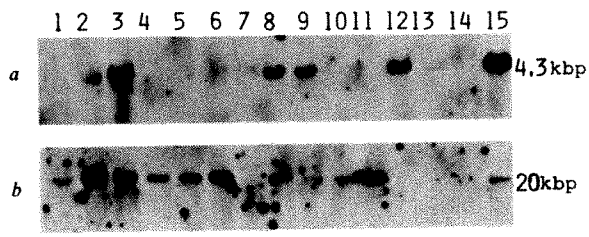


Fig. 3 Distribution of cellular sequences flanking adjacent integrated proviruses among human–mouse somatic hybrid cells containing different sets of human chromosomes (see Table 1). Hybridization of ^{32}P-labelled probes *a* (panel *a*) and *d* (panel *b*) to *Eco*RI-digested DNA from somatic cell hybrids (lanes 1–13), mouse (lane 14) and human (lane 15) cultured cells[22–24] after DNA fragments had been separated by electrophoresis and transferred to nitrocellulose filter. Probe *a* hybridizes to a 4.3-kb fragment and probe *d* hybridizes to a 20-kb fragment but neither hybridizes to mouse in these conditions. Cell hybrid DNAs either contain the human sequence (+) or do not (−), depending on the human chromosome composition of the hybrid cells. In panel *a*, probe *a*-positive hybrids are in lanes 2, 3, 7 (weak), 8, 9, 12 and 13. In panel *b*, probe *d*-positive hybrids are in lanes 1–6, 8, 10 and 11. The human chromosome constitution of the hybrids is listed in Table 1; the hybrids are listed in the same order as in the figure.

in 17 ATL cases (Fig. 2). In the *Eco*RI digests of 12 cases of ATL, probe *d* detected a 20-kb fragment covering a much wider region than the 8-kb fragment in *Hin*dIII digests, but again no rearranged fragments were found (data not shown). These results also support the findings with probes *a*, *b* and *c* described above (Fig. 1).

The above conclusion is consistent with reported restriction patterns of provirus DNA in leukaemic cells from 78 ATL patients[11,13]. Thus, the provirus integration of HTLV into ATL cells seems to differ from that in tumours induced by avian leukosis virus[14–16] or mouse mammary tumour virus[19,20], which possess one or several common loci for provirus integration.

However, it is possible that the provirus integrates into many chromosomal sites on the same chromosome. To test this possibility, we analysed the specificity of provirus integration at the chromosomal level by using the somatic cell hybrid strategy for mapping human genes on specific chromosomes[21]. Human × mouse somatic cell hybrids characterized for their human chromosomal content were analysed for probes *a* and *d*. DNA was extracted from these hybrid cells and separately analysed by the two probes on the same filter (Fig. 3). The chromosomes in which the proviruses are integrated segregated independently in cell hybrids (Table 1), demonstrating that integration occurs on different chromosomes. Analysis of the distribution of the probes in the cell hybrids tested in Fig. 3 demonstrates that probe *a* co-segregated with chromosome 7 and probe *d* co-segregated with chromosome 17 (Table 1). With the small number of cell hybrids tested, the evidence strongly suggests that the sequences recognized by probes *a* and *d* are located on chromosomes 7 and 17, respectively. Results from 15 additional hybrids tested similarly, support the chromosome mapping evidence in Table 1 (data not shown).

The absence of chromosomal specificity of the provirus integration between two ATL patients is consistent with the absence of a common integration site shown by blotting analysis of the provirus sequence in leukaemic cells. These data strongly suggest that provirus integration in tumour cells occurs at random sites.

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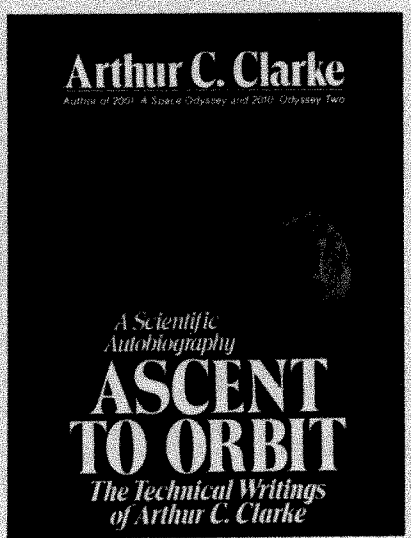
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Father, mother and son

Peter Kemp

H.G. Wells: Aspects of a Life. By Anthony West.
Random House/Hutchinson: 1984. Pp.405. $22.95, £12.95.

H.G. Wells: Aspects of a Life opens bizarrely not with the birth of its subject but the birth of the biographer — Anthony West, Wells's illegitimate son. At first startling, this emerges as rather appropriate, since, for most of the book, West's technique is to superimpose his own situation upon his subject-matter. Ultimately, it comes to seem, the aspects of a life being illuminated here are not so much Wells's but West's.

Even structurally, the book throws West into unexpected prominence — not only starting with his birth but ending at the moment when he resolved to write his father's life. The way he has gone about this is idiosyncratic, too. Most attention is devoted to the numerous women with whom Wells was involved, West's mother — the writer Rebecca West — attracting a particularly long and loathing commentary. Savage disparagement is further lavished on literary figures whose paths crossed Wells's, especially Henry James, George Bernard Shaw and George Gissing.

West's unusual handling of his material means that the real shape and substance of Wells's life get obscured — especially the trajectory he followed from a dismally disadvantaged background to fame as a novelist then descent into the role of wordy opiner on world affairs. Fuelling that progress was fierce energy. Born to parents just subsisting in a floundering little shop, Wells seemed destined — like his brothers — to life as a draper's assistant. What saved him was science. Angrily and admirably elbowing his way to an education, he eventually won a scholarship to the Normal School of Science. Here, he was taught by T.H. Huxley — who influenced him in two crucial ways. He instilled into him the importance of taking a biological view of life: something that gives Wells's writings — despite their surface diversity: fiction, Utopias, encyclopaedias, biography, autobiography, polemic and propaganda — an essential unity. And his career showed Wells what might be achieved by pugnacious advocacy of scientific training.

Initially, Wells's educational impulse took him into school-teaching. Ill health ended this. But, when he began writing, the impulse stayed with him: didactic hypotheses are fruitfully yoked to imaginative excitements in the early scientific romances — *The Time Machine, The Island of Doctor Moreau, The Invisible Man, The War of the Worlds, The First Men in the Moon* and so on — that established him as an intriguingly new kind of creative figure.



From *H.G. Wells: Aspects of a Life*

Huxley's student — H.G. Wells at the Normal School in South Kensington.

Strangely, when it comes to conveying the nature of Wells's achievement, West concentrates on his inferior, post-1914 writing. Preferring the journalist to the novelist, he lauds the bombastic and ephemeral newspaper work turned out — indeed, churned out — when Wells was straining to establish himself as a kind of global guru. Given more perfunctory treatment is the period of real brilliance in Wells's career — his first two decades as an author: when he turned the dreary and debilitating events of his early life into bracingly resilient comic fiction, and channelled the intellectual energies generated by his studies with T.H. Huxley into powerful, pulsing science fiction. Confusing notoriety with notability, West appears most impressed by the later Wells — "a world figure whose name was known wherever books were printed and newspapers were sold", "a very big man indeed".

Besides beating the drum for this strutting public figure, West is concerned with putting an attractive gloss on Wells's private affairs. It is here that his book becomes most extraordinarily personal. The idea of writing his father's life came to him, West reveals, when he was brooding over the fact that someone seemed to have lied to Wells about him, alleging that he belonged to a pro-Nazi conspiracy. Appalled at "The wilful interpolation of a piece of fiction into the facts of my life", West was struck with horror at the thought that just such another "nightmare cobweb of lies" might be draped across Wells's biography. Accordingly, he resolved "to devote the necessary time and effort required to keeping the record of what my father had been, and of what he stood for, more or less straight".

Crooked records are an *idée fixe* in this book. West finds doctored documents everywhere. His grandmother apparently concocted an "appalling fabricated diary". His mother, he claims, bequeathed to posterity a trumped-up tissue of falsifications about her relationship with Wells — drafts or summaries of letters that never existed, invented incidents, fake quotations. Another woman novelist who became Wells's mistress, Dorothy Richardson, is likewise sourly indicted of compulsive mendacity about their affair. Even a male friend of Wells's, the novelist George Gissing, gets denounced as a "veiled and secretive fraud", an obsessive "fabulist" for whom "the transformation of the truth was the name of the game".

All of this needs viewing sceptically. It is, it transpires, a habit of West's to assail people for doing what he does himself: at one point, with the shattering imprudence of the stone-thrower in the glass-house, he even lobs abuse at someone for "producing studies of the lives of dead writers". Regularly, he protests about the snobbery often shown towards Wells — while at the same time indulging himself in a very socially sniffy tone, turning up his nose at "provincials who lived in a provincial backwater", "second-raters" in "the obscurer colleges of the older universities", and Dorothy Richardson's "not very dazzling receptionist's job". Constantly, people are rapped over the knuckles for taking hearsay on trust. Yet this is West's habitual procedure: most of his story of Gissing's relations with his se-

cond wife, Edith, for instance, consists of West's account of Wells's account of a doctor's account of Gissing's account of his dealings with Edith — the resulting hodge-podge being, unsurprisingly, grotesquely at variance with Gissing's direct record of events in his diary. Quotations from Gissing's letters are scrambled too — up to five distortions in six lines — giving a misleadingly unpleasant slant to things: he's made to refer to his son as "it" not "he", his wife as "the woman" not "the poor woman".

What seems to be happening here is that facts about Gissing's life are being slid around until they fall into a pattern fitting West's preoccupations. Ferociously, West clamps the complex of feelings stemming from his lonely and divided childhood around other people. In his *Experiment in Autobiography*, for instance, Wells recounts how as a boy, when tossed playfully into the air by a youth teasing him with "Whose little kid are you?", he fell and broke his leg. This was, he observes, actually a lucky break: confined to bed and plied with delicacies and books by the youth's mother, he acquired that appetite for reading and knowledge that, via his exhilarating educational experiences studying biology with Huxley, eventually launched his career: "Probably I am alive to-day and writing this autobiography instead of being a worn-out, dismissed and already dead shop-assistant, because my leg was broken". West's interpretation of events is strikingly different: the youth, he believes, "had really put him on the spot with his question: Whose little kid was he, his father's or his mother's?"; he "had been asking him to take sides" and "The probability is that he had been trying to blurt out his father's name when he was dropped", so "It must have seemed as if he had been punished for wanting to be his father's boy".

There is, in fact, nothing in Wells's account to warrant the assertion that he was being asked which parent he preferred, or that he "had been terribly torn between being his mother's boy or his father's". West, however, has spent his life caught in the tensions of such a pull — as his novel *Heritage* about his filial fight against Rebecca West makes only too clear. His new book seems one more round in the psychic tug-of-war between what he calls in a sadly revealing phrase "the father of my dreams", and the mother whose accounts of other people "however sharply detailed and however plausible in their *trompe l'oeil* literalness, are expressive of her states of feeling rather than descriptions of actual . . . occurrences". Ironically, on the evidence presented here, West establishes himself not so much as his father's champion as his mother's son. □

Peter Kemp is Senior Lecturer in English at the Middlesex Polytechnic, London, and author of H.G. Wells and the Culminating Ape *(Macmillan, London, 1982).*

Processes of natural waters

J.D. Burton

Principles of Aquatic Chemistry.
By François M.M. Morel.
Wiley: 1983. Pp.446.
£42.55, $49.95.

THE study of chemical processes in natural waters tends to be pursued in compartments — oceans, lakes, rivers, groundwaters and so on. One reason for this lies in the large differences between such environments regarding the spatial and temporal frameworks within which geochemical processes can operate. Another is the contrast in ionic strength between saline and fresh waters, which to some extent imposes different strategies upon the application of both analytical techniques and physicochemical concepts.

Nevertheless, underlying the diversity of geochemical phenomena in natural aquatic systems is a limited number of physicochemical processes which also underlie the chemical behaviour of constituents in treated waters and aqueous wastes.

Professor Morel's textbook is the latest of several which have presented the chemistry of natural aquatic environments in the context of basic physicochemical principles. The first three chapters give a concise and clearly developed introduction to solution chemistry. The subsequent treatment of natural water chemistry is organized along lines dictated by the main types of reactions which have to be considered: acid–base chemistry, solubility equilibria, complexation, redox chemistry and surface chemistry. The tone is firmly didactic and numerical examples are widely used. Any such approach must be rooted in equilibrium systems but the importance of kinetic factors in determining what happens in the environment receives appropriate emphasis. The dominance of inorganic topics largely reflects the relatively backward status of attempts to apply physicochemical concepts to the behaviour of organic compounds in natural waters.

The author says that sometimes he has used "a cumbersomely detailed algebraic and numerical development" but this will be considered an advantage by many students trying to make the transition from description to a more rigorous approach. They will be assisted also by the frequency with which the text turns to the processes as they operate in nature, topical matters such as photochemistry and hydrothermal processes at ridge-spreading centres being included.

The material covered is inevitably similar to that dealt with in the well-established text by Stumm and Morgan, also published by Wiley, which went into a new edition in 1981. The present book has its own virtues, however, and should join *Aquatic Chemistry* on the desks of workers in the field. It is to be hoped that a cheaper edition may soon make it more accessible to students. □

J.D. Burton is Reader in Chemical Oceanography at the University of Southampton.

Yeasts and industry

Julius Marmur

Yeast Genetics: Fundamental and Applied Aspects.
Edited by J.F.T. Spencer, Dorothy M. Spencer and A.R.W. Smith.
Springer-Verlag: 1984. Pp.533. DM148, $57.50.

IN 1970, Dr G.R. Fink offered the advice that "biochemists as well as geneticists should use only S288C or 'isogenic' *Saccharomyces* strains in their investigations. Regrettably, this has not been the rule in the past". True, the principal yeast of industrial importance is *S. cerevisiae*. However, there are over 400 yeast strains, harbouring a vast resource of genes encoding enzymes that carry out a variety of chemical transformations. What is very much needed is the comparative genetic and biochemical characterization of some of the more versatile strains, making use of classical and modern genetics.

J.F.T. Spencer and his colleagues have assembled a book on the principle that "the methods of yeast genetics have many things to offer the breeder of industrial yeast strains". Thus, there are chapters on topics ranging from gene conversion to the breeding of brewing strains. While some subjects, such as gene conversion (dealt with here by Fogel, Mortimer and Lusnak) are handled in exquisite detail, others, nucleic acid relatedness among yeasts (Kurtzman, Phaff and Meyer), for example, are covered in a broad-based manner, bringing together a great deal of information that is otherwise hard to come by.

In addition to those subjects already mentioned which are "concerned with the nuclear yeast genome", there are chapters on the yeast cell division cycle (Carter, Piggott and Walton), meiosis and sporulation (Dawes), and radiation sensitivity and repair (Game). Particularly good is Elizabeth Jones's wide-ranging contribution on the genetics and biochemistry of yeast proteases and their inhibitors.

Extrachromosomal genetic elements are covered in three chapters, two of them on mitochondria. One would have been sufficient; the longer one (Evans), while initially intimidating, makes informative reading. Another contribution deals with

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Grunstein, M. and D.S. Hogness. 1975. PNAS. 72(10):3961-3965

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yeast killer toxins (Mitchell and Bevans) encoded by duplex RNA. The extensive discussion of yeast mitochondria in the book could be justified by an as yet ill-defined role of these organelles in affecting flocculation, sporulation and gemination as well as sugar fermentation.

The industrial aspects of yeast are covered in contributions dealing with protoplast fusion (Freeman and Peberdy), genetics of wine yeasts (Snow), flocculation (Johnson and Reader) and in an excellent article by Kielland-Brandt and co-workers on genetic approaches (principally their own) to brewing yeast improvement. These chapters reiterate the message that the genetic analysis of industrial yeasts suffers from their poor mating ability, poor sporulation and poor spore viability. Snow's article briefly introduces an approach that is sure to be exploited, namely, the introduction of a heterologous gene (in this case the bacterial *Lactobacillus* gene encoding the malolactate enzyme) into yeast to modify or increase alcoholic fermentation. To this end the recent introduction of bacterial genes into yeast is notable, for example the gene from *Escherichia coli* coding for xylose isomerase which provides an important step in the hydrolysis of the plentiful supply of xylans.

Finally, the volume includes a chapter by Rank and Robertson on the composition of yeast plasma membranes (somewhat out of place in this book) and a broad review of yeast carbohydrate metabolism by Steward and Russell. What is missing is a chapter on recombinant DNA technology and sections with more information on excretion of extracellular enzymes and proteins.

The contributors are principally European with a sprinkling from North America. References are mainly up to 1981, with a few reaching into 1982. Although it is apparent that the genetics of industrial yeasts is just beginning, the chapters dealing with them do bring us up to date on what is known, often reviewing literature that is obscure to most molecular geneticists. And who knows: maybe some industrial yeast will become the S288C of the fermentation industry. □

Julius Marmur is in the Department of Biochemistry at the Albert Einstein College of Medicine, New York.

Second editions

● Volume II, *Animals*, of T.W. Goodwin's *The Biochemistry of the Carotenoids*. (Publisher is Chapman & Hall/Methuen; price is £25, $50.) The second edition of Vol. 1, *Plants*, was reviewed in *Nature* **293**, 494; 1984.

● *Macromolecules*, Vol. 1 *Structure and Properties* and Vol. 2 *Synthesis, Materials, and Technology*, by Hans-Georg Elias. (Publisher is Plenum; prices are Vol. 1 $65, £50, Vol. 2 $95, £73.15.) The new English-language edition is a translation of the German fourth edition. The original English edition was reviewed in *Nature* **270**, 546; 1977.

Junior prometheans

David J. Rose

The Man-Made Sun: The Quest for Fusion Power.
By T.A. Heppenheimer.
Little, Brown: 1984. Pp.347. $19.95.

In King Arthur's legendary domain, the Knights of the Round Table slew dragons and rescued fair maidens; only the pure of heart could hope to glimpse the Holy Grail. Heppenheimer likens the quest for controlled fusion to such an enterprise, but one pursued by more fallible human beings. In a gossipy book, he takes us through many activities and events, mainly in the United States, telling his story as a sort of passion play, even to the extent of including a list of 42 dramatis personae. It is an unbalanced work, divided into 12 relatively separate acts which are often named imaginatively ("Plasmas on the Potomac") but sometimes not so neatly ("Arms and the Man"). This is a nuisance for the reader, because each chapter has no breaks or subheadings of any kind, and nothing much in the way of a summary. An index is included, but no table of contents. Thus it is a hard book to read seriously, despite the conversational style.

Behind the apparent and sometimes real clash of personalities described by Heppenheimer, there were fundamental differences about how controlled fusion research should be done. Consider the course of events in Washington between 1980 and 1982 ("The Face Off"), leading to the resignation of Edwin Kintner, Director of the Office of Magnetic Fusion Energy, which was apparently triggered by the Office of Management and Budget's insistence that $42 million — about 10% of the annual budget — should be shifted from new construction to operations. Although he fails to dig out all of the details, Heppenheimer gets the story mostly right — that the background issue was whether the time had come for proceeding more rapidly with larger test devices (Kintner's view), or whether then-current programmes should be better supported, to lay a stronger foundation for whatever might come (OMB's view).

A passionate argument lay behind this affair, which Heppenheimer did not fully uncover in his discussions with any of his 57 interviewees. It was this: what constitutes appropriate science and engineering in the daunting task of confining a nuclear fire with purely ethereal bonds, and getting cheap power out of it. Programmes and budgets refer to milestones, but the only accurate ones are on the road behind; no one knows how many lie ahead or where the end will be.

The search for fusion brought the whole glorious field of plasma physics to its maturity, but all of that science does not necessarily carry us into the world of application. The research devices of today, marvels of engineering in their own right, may not be practicable when scaled up: can the grams of tritium fuel be regenerated and recovered from tons of (probably solid) moderator? Will the vast vacuum wall, thinner in proportion than an egg-shell, survive under neutron irradiation more intense than that in the core of a fission reactor, without leaks, for years? The structure will be radioactive and require remote maintenance more complex than do fission reactors. Other engineering difficulties exist, just as severe. At the end of it all, are fusion reactors likely to be made at a competitive price?

A characteristic of the whole fusion programme has been its dominance by the plasma physicists. But the quest for fusion power involves much more than science alone, and both workers and patrons came to expect too much, too easily, too soon. Robert Hirsch, Director of US Magnetic Fusion from 1972 to 1976 (to whom Heppenheimer devotes an entire chapter), managed to increase the budget from $20–25 million to twenty times that amount, matching the scale of work to the task much better than before. But Hirsch left an unwelcome legacy — time-tables for the development of fusion reactors that presumed a state of scientific and engineering knowledge that just did not exist.

Kintner's agony had its roots in much the same sort of optimism. He advocated a series of ever larger experiments, as engineering test-beds. There were — and are — serious differences of opinion about whether that was the best way to proceed. Some engineering wisdom comes from such billion-dollar attempts, but does it come too narrowly, at too great a price? Other problems need working out more generally, and the answers are essential in helping us to judge the feasibility of fusion before resources become committed to a single giant device.

Heppenheimer devotes two chapters to laser fusion, starting with the late Keeve (Kip) M. Siegel, founder of KMS Fusion, and his chief scientific associate, Keith Brueckner. In the laser approach, a sub-millimetre pellet containing a deuterium-tritium mixture is subjected to a huge laser pulse that blows off the outer layers of the pellet, compresses it and heats it to fusion temperature. That scheme is related to how an H-bomb works. The somewhat delicate point arose whether Brueckner, who had some access to the technology, was using government-financed classified research for private commercial advantage. Apart from covering such tittle-tattle, Heppenheimer discusses this whole approach to fusion optimistically, an attitude that might find its explanation in the list of enthusiasts he interviewed.

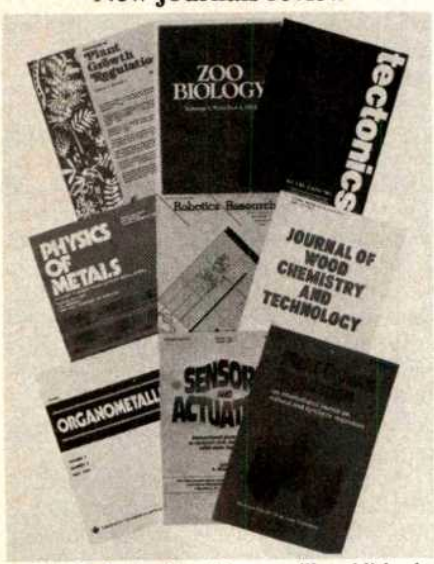
This kind of star-wars view of fusion energy (and perhaps of fusion research in general) seems to me to lose sight of the original goal: to provide a rather mundane

but essential service — energy — in as simple ways as are consistent with economic, environmental and social goals. Continuing experimental and analytical studies of laser fusion seem to be raising the technological ante for success faster than budgets, technology, and perhaps even science, can cope.

As to the characters and events themselves, Heppenheimer sometimes tells us too much — nine pages on a fight about whether an advance in achieved plasma energy was to be publicized, for instance. Are we really so interested in the appearance of the last part of Sir Galahad's horse to leave the stable? But in his last chapter, and occasionally elsewhere, Heppenheimer catches the spirit that inspired the pioneers in this enterprise. They were among the most brilliant of scientists, both in the United States and elsewhere, and did what they did because it was, in some deep way, a good thing for society to do. Some of that spirit still exists; without it, the quest will surely fail. □

David J. Rose is a Professor of Nuclear Engineering at the Massachusetts Institute of Technology.

New journals review



On 27 September *Nature* will publish the fourth annual review supplement devoted to science journals.

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A different sort of Darwinism

J. H. Brooke

Darwin and the Spirit of Man.
By Alister Hardy.
Collins: 1984. Pp.245. £9.95.

SIR Alister Hardy is prominent among those who have argued for "Darwinism with a difference". Whilst acknowledging the creative powers of natural selection, he enshrines innovative behaviour as a distinctive selective force. His argument is that a learnt and beneficial habit can be instrumental in structural selection by favouring *subsequent* genetic change which might reinforce the behavioural novelty. In the approved words of R.F. Ewer: "behaviour will tend to be always one jump ahead of structure and so play a decisive role in the evolutionary process".

From such a possibility, Sir Alister advances the claim that behavioural selection has been one of the "major factors" in the evolution of birds and mammals. His sights are set on what he considers an "intellectual scandal" — the notion that Darwinian evolution can only be construed in materialist and reductionist terms. Using results from his Religious Experience Research Unit, his aim is to present an alternative synthesis which does justice both to the integrity of biological explanation and the reality of religious experience. So urgent a purpose in so distinguished a figure deserves serious consideration.

Informal in style, almost evangelistic in tone, the exposition strives for intellectual unity in a manner which strikes one as more impressionistic than formally rigorous. Even non-specialists may be perplexed by the way Sir Alister structures his alternatives. The reader is asked to choose between a chance mutation or a new habit as the instigator of adaptive change. Sir Alister's heart is with the latter, elevating the conscious, explorative and perceptive aspects of animal behaviour. But may not the choice itself be misleading? If a bird learns a new trick, even how to raid a milk-bottle, the capacity to learn was presumably already there in the gene-complex, having been encouraged by past selection. And if the new behaviour continued to be advantageous, it would presumably be favoured, again by the normal process of selection. Conversely, a mechanism whereby genetic change *subsequently* reinforced a behavioural innovation might invite the objection that it could destroy a useful flexibility of response. The new habit, if genetically and structurally "fixed", might sacrifice versatility for a precarious short-term gain.

A constrictive choice is also presented in the treatment of consciousness: either an extreme reductionism, in which consciousness is epiphenomenal; or a form of psychophysical dualism, affirmed on eminent authority but largely assumed by Sir Alister rather than specifically argued for. Excluded by this dichotomy would be those monistic accounts which treat consciousness as an "emergent" property, associated with a particular degree of material organization, but neither reducible to it nor the product of an elusive spiritual element. It is Sir Alister's view that the spiritual element is not, after all, elusive. It exists in all mankind and may be discerned in numerous reports describing a



Sir Alister Hardy — "Whilst acknowledging the creative powers of natural selection, he enshrines innovative behaviour as a distinctive selective force".

"feeling of receiving help from a power, from Divinity, beyond the self".

The perplexity here is that whilst the author offers a sensitive classification of reported experiences, it is not shown how, from reports alone, one can establish anything further than the point that such feelings of dependence are common. The counterpoise is still that which weighed so heavily with the young Charles Darwin as he studied the natives of Tierra del Fuego: such feelings were apparently not shared by, and therefore not essential to, all humanity. The further suggestion that this sense of drawing upon a power outside the self might have become an energizing force in human evolution, a "something that gave more courage than [men] had ever had before", is not without difficulty. Might that sense of dependence not just as easily encourage passivity as activity? Alternatively, supposing it had gradually been reinforced by its survival value, would that really persuade us that man's spiritual nature had any transcendental significance? But whatever one's response to Sir Alister's confessedly personal view, it is stimulating to engage his own burning questions. □

J.H. Brooke is a Senior Lecturer in History of Science and Principal of Bowland College, University of Lancaster.

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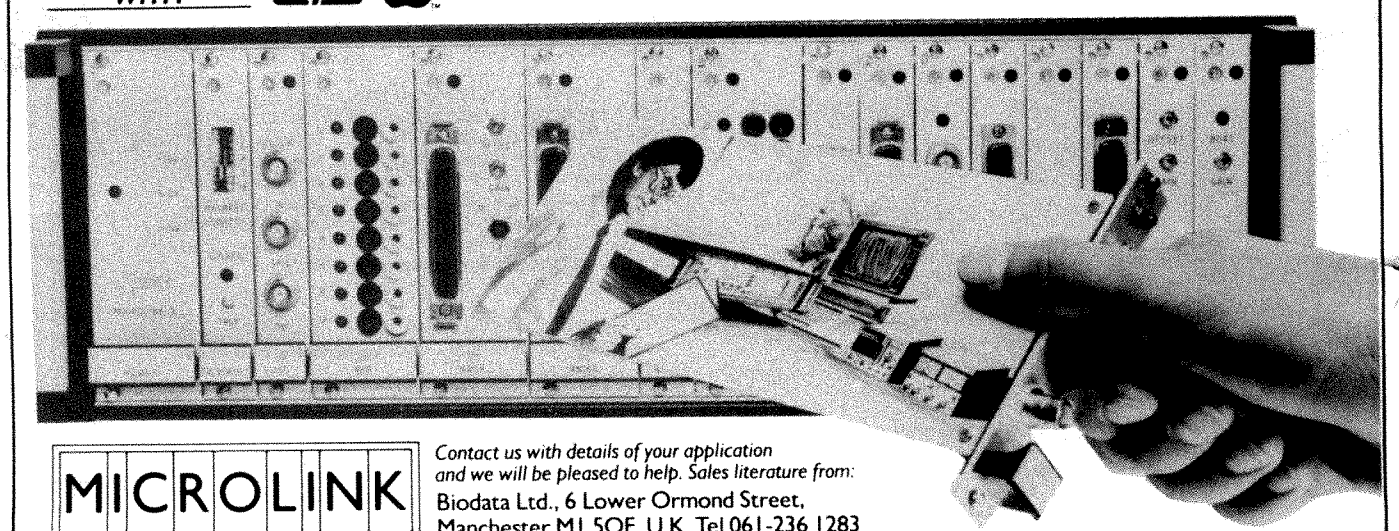
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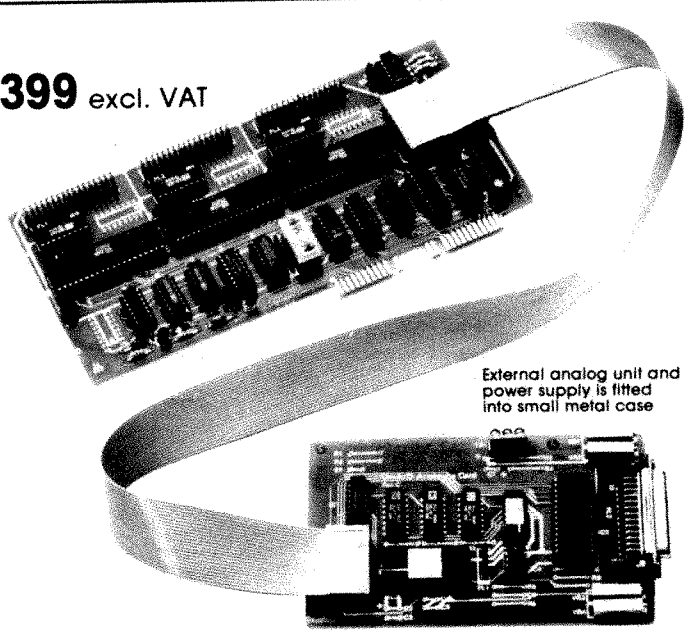
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Automation is just beginning

The laboratory application of computers is being extended rapidly, but there is some way to go before the penetration of these machines is as complete as, say, that of the Bunsen burner.

A FEW years from now, the heading on these pages will be entirely inappropriate. The idea that the use of computers in laboratories is sufficiently novel to be remarked upon will have ceased entirely to be noteworthy, just as it would now seem strange to assemble a few pages of this journal under the heading "oscilloscopes in the laboratory". So charitable readers are invited to regard the pages that follow as a reminder that the present is a transitional period, one in which it is not yet second nature for experimentalists and theorists alike to use all the tools now potentially at their disposal.

It is also worth remarking that the present hectic period during which the laboratory computer is being made familiar has its roots in a period which long predates the development of electronic computing machinery. One of the obvious illustrations of this truth is in the development early this century by C.T.R. Wilson of the cloud chamber, first for stimulating processes occurring in the atmosphere, later for the recording of cosmic ray particles by the trail of droplets produced in a supersaturated vapour. The first studies of cosmic rays were necessarily made with cloud chambers whose expansion (to give supersaturation) was carried out at random. But the technique became productive only when, in the 1920s, cloud chambers could be made to record events only when the chance of something interesting being observed was high.

The (Geiger) counter-controlled cloud chambers of those days are forerunners of the computer-controlled experimental rigs that are now commonplace in many important ways, but not least because they stimulated the development (with thermionic valves or tubes) of the logic circuits now embodied by the thousands in microprocessors. Over half a century, the practice of particle physics has been inextricably bound up with the development of electronic techniques for making sure that observations are made only when it is profitable to do so. And while the techniques have now spread to a host of other fields in which the objective is to record transitory phenomena, accelerator physics remains the citadel of the selfconscious practice of selecting from a mass of naturally arising happenings only those about which observational data are likely to be useful.

Computer-controlled experiment has the virtue of economizing in effort in the way most likely to be productive — by the avoidance of unwanted data. This is the spirit in which, in many biological applications (and more generally), it is possible to record data only for as long as necessary to provide some predetermined degree of statistical certainty. The prerequisite of such procedures is that there should be a computing facility "on-line" with the data-gathering equipment, carrying out an analysis as the experiments proceeds. Again, inevitably, the accelerator people have blazed a trail which everybody will soon be compelled to follow. For the collection of needless data is not merely inelegant but an encumbrance, a distraction. Even now, however, the point is not sufficiently forcefully made in teaching laboratories.

The notion that computers in one form or another have an important part to play in the collection ("logging") and analysis of data is, by comparison, more readily accepted. What seems to be less widely appreciated is that there are already a great many kinds of investigations now commonly undertaken that would be beyond the scope (and stamina) of people without the assistance of computing machinery. Particle physics has again been the pathfinder in this trend, with its techniques for the recording of tracks (or points along the tracks) left by particles in detectors of various kinds, and for their subsequent automatic analysis.

Inevitably, this practice has spread like wildfire. Deep seismic refraction, both in the exploration for petroleum and in geophysical investigations, generates such masses of data that only computer recording and analysis is feasible. The identification as well as the measurement of stellar and galactic objects on photographic plates collected by astronomers is similarly a way of carrying out tasks that would otherwise be impracticable. And even where the quantities of data are not nearly as overwhelming, there remains the possibility that they will not be fully interpreted because the investigator has not the stomach for the task or, more charitably, takes the view that his priorities lie elsewhere. Yet even in this uncontroversial field, where the value and the objectivity of automatic data analysis is generally accepted, teaching laboratories are not nearly as well equipped as they might be.

The application of computers as means of retrieving data is, by comparison, commonly undervalued. Many people have found the widely available bibliographical databases less than a substitute for the professional literature. And it is true that many of these systems are needlessly wooden and inflexible. There may be more promise in the schemes (some of which are referred to in the pages that follow) that assist people to construct their own individual databases, for mnemonics are almost certain to be more "friendly" (in the computer jargon) than substitutes for one's memory.

The use of databases simply for the recognition of meaningful portions of the nucleotide sequences of DNA which have become a common feature of the literature is perhaps the most telling illustration. The plain truth is that it is almost conceptually impossible for a person to compare the sequence of some nucleotide sequence he has constructed with a library of known gene sequences so as to pick out those which most closely resemble each other. Not so long ago, an author of an article submitted for publication was much chagrined that a referee with access to a better computer program was able to spot a similarity that had previously gone unrecognized. But this is only one set of circumstances in many where the full power of computer retrieval remains to be recognized. Rivalry between laboratories will no doubt bring other cases to light.

But none of this, it will be remarked, bears on what must seem the most obvious application of computers in the laboratory — the accomplishment of mathematical tasks that would otherwise be beyond the scope of ordinary people. The use of powerful computers for the simulation of, say, the evolution of weather systems requires no special justification, but the huge installations by which these tasks are accomplished are (and are likely to remain) the exceptions, not the rule.

More interesting are the applications of computer programs to ordinary tedious tasks of algebraic analysis, still regarded as a kind of frivolity or luxury, and to fields in which the underlying characteristics of a mathematical process would not otherwise be apparent. Who would at this stage claim that the importance of chaotic (or "deterministically random") phenomena would have been widely recognized without the computer simulations of the process that are now commonplace? Or that the study of fractal systems could have been accomplished analytically if only mathematicians were less idle? □

Computers in books

Microcomputing in biology

A.N. Barrett

GIVEN the shortage of suitable software for analysing biological data by microcomputer, the publication of books containing methods and programs for that purpose is generally all to the good.

One recent book of this type is *Statistics and Numerical Methods in BASIC for Biologists,* written by J.D. Lee and T.D. Lee and published by Van Nostrand Reinhold (hbk £14.95, $32.50; pbk £7.75). The authors include methods for determining means and standard deviations, chi-squared tests, various correlation coefficients, linear regression and curve-fitting. There are also two chapters on the biological applications of statistics: one on assays, the other on linear regression techniques applied to bacterial growth data.

Accompanying the methods are some 20 programs, written in elementary BASIC, which are highly suitable for laboratory use. The programs are well structured and incorporate numerous checks against incorrect or pathological data. Documentation is provided with the programs, enabling the user to follow easily the program structures, and sample data is available for users to test the programs on their own computer. One price of the robustness of the programs, however, is that they are considerably larger than similar programs performing the same calculations. And to ease typing problems, it would be useful if the programs were available on a floppy disk for the Apple and BBC microcomputers.

A book with similar statistical content is *BASIC Microcomputing and Biostatistics* by Donald W. Rogers (Humana; $39.50 (US), $49.50 (elsewhere)). The aim of the book is to teach essential programming skills, using BASIC, and to provide a range of statistical and mathematical techniques for the analysis of data in the physical and life sciences (including medicine).

The book starts with a rather brief discussion of computing and BASIC, with three simple programs illustrating certain BASIC functions. The next seven chapters deal with a variety of standard statistical techniques and also provide simple programs to illustrate the application of the techniques to limited data sets. The statistical methods introduced cover the understanding of experimental errors and the computation of means and averages, and the determination of probability distributions together with the use of the Poisson and Normal distributions. A chapter is also included on the application of the chi-squared test and Student's *t*-test. Finally, least squares are explained, as well as the fitting of nonlinear curves and the solution to simultaneous equations.

From the point of view of the laboratory scientist, one of the limitations of Rogers's book is that many of the programs are unsuitable in their present form for analysis of experimental data. The curve-fitting program, for example, only extends to a second degree curve and several of the earlier programs are restricted to the determination of properties of groups of five numbers. The principles used do not easily lend themselves to extension of the programs to handling large data sets and as such are of limited value except for teaching. In this context, however, the book achieves many of its stated aims; a lot of examples are provided and the programs are well supported by background information.

In contrast to the preceding two books, James D. Spain's *BASIC Microcomputer Models in Biology* (Addison-Wesley; $34.95, £22.10) contains few programs and concentrates on providing flowcharts to accompany the methods from which the readers themselves can develop programs. Several useful programs in BASIC are available, however — for curve-fitting, polynomial fitting, normal distributions, queuing simulation and graph plotting — either as listings contained in an appendix or on a floppy disk (price $50). The programs run on either the Apple II or TRS-80 computers. The appendix also includes an introduction to programming in BASIC.

The main text is divided into three parts. The first deals with simple equations that model single biological systems or system components such as exponential growth and enzyme saturation. The second is concerned with deterministic models of multicomponent systems and includes, for example, chapters on biochemical reaction kinetics, dynamics of homogeneous populations of organisms, models of microbial growth and simulations of population genetics. The final part deals with the effects of random processes on biological systems, with special attention being paid to sampling processes, random walks and queuing.

As a means of introducing both students and practitioners of biology to theoretical methods and their applications, Spain's book fulfils a useful purpose. Its structure will appeal in particular to those with sufficient programming skills to translate the flowcharts into working programs.

Finally, *Microcomputers and Physiological Simulation* by James E. Randall (Addison-Wesley; $27.95, £14.50) is a softback aimed at providing an introduction to microcomputers and their use in the mathematical simulation of physiological processes.

The first five of the 14 chapters present a discussion on both hardware and software, and include accounts of microcomputer components, and operating system and programming languages, together with graphics facilities for the TRS-80 and the Apple II. Each chapter concludes with a list of addresses of manufacturers of both hardware and software, where appropriate, and with references to relevant journals.

For a book dealing with techniques of mathematical simulation, surprisingly little background mathematics is included. Instead, the author concentrates on providing BASIC (Apple) programs encapsulating the relevant mathematical equations necessary for the simulations and refers the reader elsewhere for the supporting theory. The complexity of the programs varies with the particular application (axon potentials, cardiac action potentials, distortion of physiological waveforms and the glucose tolerance test are among those covered).

Randall's book clearly demonstrates that a variety of physiological processes can be successfully simulated on the microcomputer. It should be of interest to advanced students and physiologists with interests in modelling. □

A.N. Barrett is in the Computing Laboratory at the National Institute for Medical Research, London.

Computers and genes

E.G. Richards

The Applications of Computers to Research on Nucleic Acids II, Parts 1 and 2.
Edited by D. Söll and R.J. Roberts.
IRL Press: 1984. Pp.856. Pbk £30, $60.
Time Warps, String Edits and Macromolecules: The Theory and Practice of Sequence Comparison.
Edited by D. Sankoff and J.B. Kruskal.
Addison-Wesley: 1984. Pp. 382. $28.95, £24.60.

THERE are over one-and-a-half million bases in the latest edition of the EMBL nucleotide sequence library and somewhat more scattered through the literature. If printed, they would comfortably fill the two volumes edited by Söll and Roberts and totally bemuse anyone who opened the pages of such a book. It is then no small wonder that the writing of computer programs to aid the construction and digestion of nucleic acid sequences is a growth industry. The two volumes were originally published as Volume 12, Number 1 of the journal *Nucleic Acids Research* in January of this year. A similar work, half the size, was published in January 1982, giving us a doubling time of two years.

The three parts of the latest volumes are devoted to main-frame computers, mini-

computers and microcomputers. The 79 papers describe programs and systems which range from the grandiose to the trivial. Several of the papers describe large menu-driven systems for processing sequences, including printing out sequences in various formats, translating them into amino acid sequences and searching for restriction sites to mention but a few of the options. Other contributions are devoted to more specialized tasks, such as working out restriction maps, ascertaining the homology between sequences, determining the most stable base-pairing scheme in an RNA chain, calculating the molecular size of fragments observed in polyacrylamide gels and even designing your experiment for you. There is only one paper devoted to the graphic display of three-dimensional structures; this is notable for being implemented on a microcomputer.

Molecular biologists engaged in producing nucleic acid sequences or cogitating on their significance are likely to find something of interest in the two books. It may be that the author will send them a listing when asked (some offer so to do, others are silent on this point), that the program is written in a language and dialect familiar to their local computer, and that appropriate hardware is available. If all this is so, such researchers may be saved hours of work reinventing the wheel. But it is unclear whether the programs themselves have been verified in any way, and hard to tell how robust they are against misuse or how easy they are to use.

Most of the programs concerned with homology and structure prediction are based on the dynamic programming method discovered, apparently simultaneously, by several workers in the 1970s. This method, based on an argument by induction for ascertaining the minimum number of insertions, deletions or changes required to transform one sequence into another, is well described in *Time Warps, String Edits and Macromolecules.*

In this book, various contributors explain how almost identical problems arise in several disparate fields, including speech recognition. Here the pressure wave corresponding to the utterance of a word is sampled at discrete times and a resulting vector compared with that of a standard word. Since people vary in the rate at which they speak, the sampling rate must be varied in some way; that is, time must be warped.

Much of Sankoff and Kruskal's volume should be accessible to molecular biologists interested in the intricacies of sequence comparisons and similar problems, and should serve a useful role in acquainting them with the state of play in this area. Other parts are more mathematical and thus more suitable for the computer specialist. □

E.G. Richards is Senior Lecturer in Biophysics at King's College, University of London.

How to crunch your numbers

Gordon C.K. Roberts

Biodata Handling with Microcomputers.
By R.B. Barlow.
Elsevier Biosoft, 68 Hills Road, Cambridge CB2 1LA, UK: 1983. Pp.261. £18, $30. Disks £30, $50.

EVEN the least expensive "home computers" are capable of carrying out all the statistical analysis that most biologists will require — and usually before the answer can be obtained from the computer centre. The main obstacle to practical realization of this is, of course, the limited availability of appropriate programs. Behind the somewhat mysterious title (what *is* "biodata"?), this book goes a considerable way towards meeting the need. It contains some 50 programs which cover most simple statistical procedures, from standard deviations and significance tests through analysis of variance to linear and non-linear (iterative) regression.

The book contains listings of the programs (in Commodore PET BASIC), reproduced from a dot matrix printer, while the text is reproduced from typescript — both are entirely legible, although the multi-statement lines and lack of indenting make the programs harder to follow than they need be. White space is prominent, since many pages are under two-thirds full, and there are several rather un-funny cartoons.

Each program is accompanied by a brief but generally adequate commentary describing what is going on, and in many cases also by an even briefer outline of the underlying mathematics. Beginners may need a statistical text to amplify some of the explanations if, as the author hopes, they wish not only to use this book as a "toolkit" but also to understand how the tools work. In addition each program has a sample output, the examples being drawn primarily from pharmacology.

The programs cover clearly the most widely used statistical procedures, and are available on disk for Commodore PET, Apple and Commodore 64 computers. The combination of disk and book certainly adds up to a useful elementary statistical package. □

Gordon C. K. Roberts is in the Division of Physical Biochemistry at the National Institute for Medical Research, London.

Pascal in practice

Graham H. Kirby

Pascal Applications for the Sciences: A Self-Teaching Guide.
By Richard E. Crandall.
Wiley: 1984. Pp.246. Pbk £13.90, $16.95.

THE number of textbooks which deal with computer programming in Pascal is very large and still increasing. So it is refreshing to find that Richard Crandall's book concentrates on *applying* the language. The author's aim was to provide a text which would enable students of mathematics, physics, chemistry and biology to learn scientific programming in Pascal. Applications in each of these specific subjects follow a general introduction to simple numerical programming.

The book is a self-teaching guide, consisting of text, exercises, answers, more text and so on. Some exercises involve modification of the many programs presented in the text whilst others are distinctly ambitious. Many answers are short comments and hints rather than code. One of the good features is the emphasis on graphics to display computed results, rather than on the printing of tables of numbers, and a microcomputer with graphics facilities and Pascal would provide the ideal basis from which to benefit from the book. Also commendable is the provision of source code for libraries of Pascal procedures covering graphics, matrix handling, statistics and specialized mathematical functions. Of course, the graphics library is hardware specific (for Tektronix 4012 terminals).

A book such as this, modestly-priced and devoted to applications, has insufficient space to introduce the language itself. Readers will require either a prior knowledge or an introductory textbook on Pascal programming. Without one or the other, the benefits of Pascal will not be appreciated since Crandall has concentrated entirely on the mathematical modelling type of application using simple numerical and statistical techniques. This is a pity because the full power of Pascal is not demonstrated — I noted very few occurrences of the type *char*, only one of records (for complex numbers) and of the case statement, and none of pointer types. The programs could be re-written in FORTRAN or BASIC with few changes.

Science students will find this book helpful in learning to write simple Pascal programs for applications in their subject area. I hope they go on to learn more of the facilities in Pascal; many scientific applications do require data structures other than arrays and types other than integer and real. □

Graham H. Kirby is a Lecturer in Computer Studies at the University of Hull.

● Reston/Prentice-Hall have just published *Scientific Pascal* by Harley Flanders, a book written specifically to teach practical programming to scientists and students of science. Price is hbk $29.95, £28.45; pbk $21.95, £20.85.

Sorts of references

K.V. Roberts

PAPERBASE: A Computerized Personal Database for Scientists.
By Anthony Durham.
Wight Scientific, 44 Roan St, London SE10 9JT. Booklet, pp.31, £25, $50 (airmail postage included) for institutional use; £10, $20 for personal use. Diskettes available for various computers, £50, $100 (booklet included).

PAPERBASE is a BASIC package for constructing and interrogating a database of references. It normally uses a microcomputer with diskette drives, screen, keyboard, printer, BASIC interpreter and word-processing program, although it can run on larger or smaller systems. I tested it on an IBM PC-XT and found the increase of speed provided by a Winchester disk and the BASIC compiler to be an advantage, although it is still a very useful system without these facilities.

References and keywords are input using program STORE: it is important to get the format correct, but this is not difficult and one can edit the ASCII output file later (for example to add new keywords). ORDER sorts alphabetically by first authors and an on-line search on logical combinations of expressions is carried out by SEARCH, producing a file containing those references that meet the given criteria. References are converted to the format required by a particular journal by LAYOUT, using word-processing facilities such as boldface and underlining where appropriate. The user may need to develop his own format code since journal practice differs so widely, but examples are given and it should not take long to master the rules. Finally, HUNTER scans through an ASCII file — for example a typescript produced on a word-processor — to report the references that it finds.

The BASIC program may have to be modified before use, depending on the computer. For the IBM PC no changes were necessary and PAPERBASE ran straightaway. However, the code is well documented in the booklet, and advice is given on how to make changes should these be needed. Users may also wish to extend the programs to meet additional requirements or to build them into larger systems; I found it helpful to modify SEARCH so that it did not distinguish between upper and lower case, and this was readily done with a few BASIC statements although it somewhat slowed down the search.

Altogether PAPERBASE is a well-engineered piece of software. It should find wide use throughout the scientific community. □

K. V. Roberts is Head of the Theoretical Physics Division at the Culham Laboratory of the UKAEA, Abingdon, Oxfordshire.

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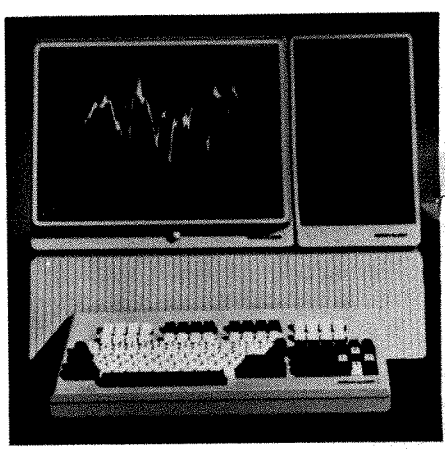
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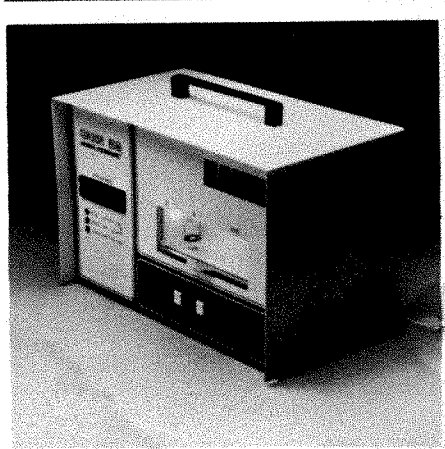
● Beckman Instruments' SYNLIB is a computer software system for the creation, maintenance and search of chemical reaction data files. The SYNLIB program has been designed specifically for synthetic chemists involved in the manufacture of organic chemistry products, pharmaceuticals and chemical specialty products. The SYNLIB system is distributed with a database of over 12,000 chemical reactions abstracted from the scientific literature. A continuing database updating service is available, and users may easily establish, maintain and search their own chemical databases containing chemistries which are of local interest.
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● TUTSIM is a program for the simulation of contaminous dynamic systems, and is now available in a short form which is suitable for evaluation, demonstration and instructional study. TUTSIM is available for IBM XT/PC, and PC jr, Apple II and some CP/M machines. The TUTSIM program was created by the Twente University of Technology in the Netherlands. It is a block diagram orientated program which produces both numeric and graphics output. Disciplines to which TUTSIM is applicable include physiological and biological model studies, thermodynamics and fluid dynamics and econometrics and sociological systems. TUTSIM is available in the United States from the Applied i company of the Palo Alto, California.
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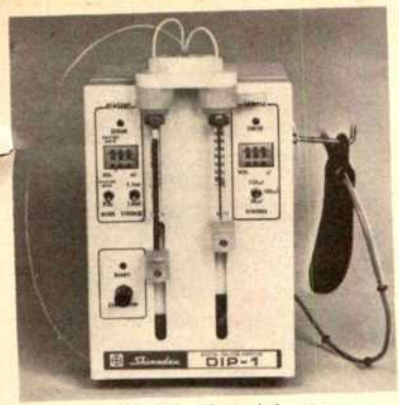
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● Technical Database Services (TDS) of New York is offering a new search and retrieval service for the Log P Database. Subscribers at remote computer terminals can search over 25,000 records containing partition coefficients and related information for over 12,000 organic compounds in approximately 300 solvents. This compilation of Log P data results from a cooperation between the Pomona College Medical Chemistry Project and scientists throughout the world. The database, now in its fourteenth year, is updated semi-annually and increases by approximately 2,500 records each year.

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● The Shimadzu DIP-1 is a simple-to use computer controlled dilluter/pipetter. Just set the dilution ratio on the digital dial, selectable in 18 steps of 2 to 100, and it will accurately dilute the sample at that ratio on dispensing. The unit can also be used for dispensing, sampling, and diluting-dispensing. When only a reagent is to be dispensed, if the set volume is larger than the full stroke volume of the syringe it will automatically repeat to provide the desired volume up to 9.99ml. When an extremely small sample is to be taken (5 to 25μl) the volume required is set on the digital dial, and the tip of the nozzle placed in the sample. At one press of the "start" button the volume of the sample will be drawn up, and when pressed again the sample will be expelled into a container. When used for diluting-dispensing, the volumes of reagent and sample are set, and all the rest is performed at a press of the "start" button.

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● AUTOSCAN, from Spectra-Physics is a BASIC program on a PROM chip for automatic stop-flow peak scanning, absorbance ratio determinations, and time-programmable wavelength changes. The program is intended for use with the SP8440 UV/VIS variable wavelength detector in conjunction with Spectra-Physics HPLC systems and the SP4200 computing integrator. In addition to reporting absorbence ratios and plotting of spectra, AUTOSCAN allows for completely unattended operation since the HLC pump start/stop functions are integrated into the program software. The chip plugs directly into the memory board of the SP4200 computing integrator and is the latest addition to Spectra-Physics Special Purpose Software Programs $(SP)^3$ series. This firmware-based system eliminates the need for loading programs from auxiliary storage devices such as disk or magnetic tape.

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● A Thermex interface converts Apple II Plus or Apple IIe computer into an automatic-printing 16 channel thermometer. The interface uses J, K or T thermocouples as sensor, and resolution in the biological temperature range is 0.1°C. At other temperature ranges an accuracy of 0.28% full-scale is achieved using individually calibrated thermocouples. A special calibration program is available, allowing the user to calibrate and linearize thermocouple probes in any desired range. Thermex, available from Columbus Instruments of Ohio, has built-in cold junction compensation. Biological probes for rectal, intravenous and implantable applications are available for animal use. A video monitor displays current temperatures in all channels. Data are also periodically printed and saved on disk at selected intervals. An optional data-transfer package allows information to be transferred from disk to files to a host computer via an RS-232 interface.

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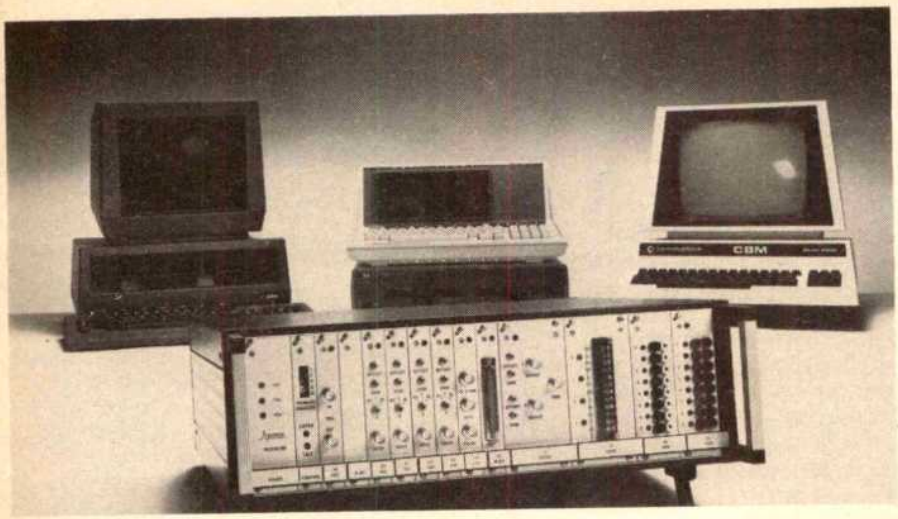
The Radiometer D470 (top), the AMICA titration system (centre) and a thermometer for use with Apple computers

● Routine photometric measurements, potentiometric titrations of all kinds and photometric titrations can be performed with the computer controlled AMICA system from Hamilton. The titrimetric methods used include acid-base titrations, redox titrations, titrations in non-aqueous media photometric titrations with or without indicator and complexometric titrations. Because of its modular design an AMICA system can be adapted to the individual needs of any analytical control laboratory, whether the need is a small number of samples and frequent change of method, on many samples for few models.

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● ELISA-CALC, available from Comple-Software of Iowa City, is designed to transfer data from an automated ELISA plate reader to a personal computer. The data are then saved on floppy diskettes; each diskette will hold data from more than 500 plates. ELISA-CALC allows the user to enter a custom plate "map" defining the contents of each well on the ELISA plates; this map is saved on the diskette so that it can be used indefinitely. The computer displays graphically each standard curve, either as a semi-log or as a log-log plot. It then allows the user to pick the appropriate points to produce the best line to fit each curve. Then it calculates each unknown on the plate using the appropriate standard designated on the plate map. It allows the user to calculate ELISA data immediately after the plate has been read, spending two or three minutes rather than hours per plate. ELISA-CALC is available to run with automated ELISA readers sold by Bio-Tek Instruments, Inc., Dynatech Laboratories, Inc. and Flow Laboratories. Inc.

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Biodata's Microlink interfacing system

● The CmC BUSSter RGA is a microprocessor-based interface designed to give RS-232 computers and terminals the ability to function as IEEE-488 controllers. Up to 15 individually addressed IEEE-488 devices can be connected through the BUSSter RGA to one RS-232 port. The BUSSter RGA serial port features include switch-selectable band rates (300 to 19,200), parity and word length. It can be configured for talk and listen commands for any IEEE-4888 address or secondary address from 0 to 31. Multiple devices can be addressed at the same time. The RGA is also compatible with service request, serial poll, parallel poll, remote/local, local lockout, device clear, and device trigger interface functions.

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● Coulter Electronics have now introduced into Britain the Micromeritics computer-controlled DigiSorb 2600 gas adsorption analyser. This completely automated physisorption instrument may be used with a wide variety of adsorbates to determine surface area, pore volume, pore size and pore area. Features include operator selectable parameters, automatic pressure transducer calibration, sample outgassing to 550°C and a large liquid nitrogen reservoir. Unattended operation is possible for up to 4 days. Automatic data and graphic presentations are printed for up to five samples.

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● The Philips Automated Laboratory Management (PALM) system can tackle both sample analysis control and industrial proces/quality assurance. A typical PALM installation might include automatic sample preparation machines with conveyor links to a laboratory or directly to an X-ray spectrometer. The PALM system is principally intended for use in conjunction with Philips equipment, but instrumentation other than Philips may be used. Results from various sources can be merged to give a complete analytical report with a minimum of delay, via terminals or printers in the laboratory or at remote locations.

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● Integration of chromatography data can be achieved swiftly using Autovideogration software for the Apple IIe or II+ computer. The program, from Heyden Datasystems, can be used with thin layer chromatography as well as being applicable to many GC and LC needs, especially when fewer than 20 peaks are being measured. Raw data may be viewed on the screen with scale expansion. Baseline position is under user control and integration may be automatic, manual or peak by peak. Results are tabulated with percentage peak areas, and all data can be stored to disk for subsequent examination.

Circle No. 116 on Reader Service Card.

● Two new features for GenBank, the genetic sequence data bank from Bolt Beranek and Newman Inc. (BBN), make for improved accessibility. A new interface allows the user to access GenBank data via a short series of menus and dialogues, and access is now possible through Telenet, an internationally available communications network. This eliminates the need for dialing into the system via long-distance telephone lines. GenBank, developed by BBN and Los Alamos National Laboratory for the National Institute of General Medical Sciences of the National Institutes of Health, is a repository of all published nucleic acid sequences greater than 50 nucleotides in length, catalogued and annotated for sites of biological interest.

Circle No. 117 on Reader Service Card.

● The publishing company Elsevier has introduced a range of scientific software with source code listings. The programs now available are: REVALUE: a software package to calculate reference intervals from total hospital patients laboratory date (for HP 85, PDP 11/23, PDP 11/45, PDP 11/70, VAX 11/780); BALANCE: a program to compare two series of measurements (Apple, IBM PC); and INSTRUMENTUNE-UP: an experimental optimization program, which helps the user to improve the performance of common scientific laboratory instruments (Apple, IBM PC).

Circle No. 118 on Reader Service Card.

● Electroplan of Royston, Herts, specializes in interfacing analog and digital signals from scientific instrumentation and laboratory experiments into many types of desk-top microcomputers. Systems available include Biodata's Microlink, which consists of a card frame into which modules are slotted to build up a flexible interfacing unit. Microlink connects to computers using the standard IEEE-488 interface, and interface cards and programming software are available for use with Hewlett-Packard, IBM, BBC, Commodore CBM PET, ACT Sirius and Apple computers. Electroplan is also UK distributor for Tecmar Labmaster and Labtender, which are data acquisition products designed to plug directly into the IBM-PC and other compatible computers. These are multifunction cards, offering 16 analog inputs, a 24-line general purpose digital input/output interface, and 5 event counters and timers on one convenient plug-in.

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● Beckman's products for laboratory information processing can be divided into three categories: data acquisition; data analysis; and sample, test and method and result management. Data acquisition includes intelligent instruments, RS-232 interfaces, standard information protocols and interfaces for other manufacturers' instruments. Data analysis includes application-specific software packages for such applications as tablet dissolution, kinetics, content uniformity and gel scanning analysis. Sample, test and method and result management includes large-scale systems for automating entire laboratories. Beckman systems interface directly to all laboratory instruments from all major manufacturers. Both instruments and accessories can be controlled. The systems are directed by laboratory-developed methods. MK 4 and MK 5 Digimetry instrument couplers provide for interfaces with analog instruments, as well as instruments without an RS-232 interface or standard information protocol. Data acquisition programs can run on personal computers, as well as large systems. Beckman's systems provide extensive data analysis for general chromotography, simulated distillation, gel permeation, continuous flow and potentiometric titration. For sample, test and method and result management, the database is determined by the laboratory and updated on-line. The systems provide a configurable operational sequence and configurable sample and test status requirements according to the laboratory's specifications. The systems feature long-term archiving and recall. Beckman systems can communicate with other CALS systems, personal computers and mainframe computers.

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Biotechnology manpower in the UK

from Richard Pearson

The best brains will not remain in this country unless the best jobs are here too.

BIOTECHNOLOGY is one of the fast growing new technologies. It was born as late as the 1970s and is recognized everywhere as a key technology for the future. As well as the United States and Japan, the technology superpowers, France, West Germany, Switzerland and the United Kingdom have taken initiatives to develop their biotechnology, even though the industry has barely moved out of the fundamental research stage. Being a knowledge-based industry, the availability of skilled manpower is crucial to its future success. In the United States, the Office of Technology Assessment has assessed prospects for that country and also provided an international overview, and has highlighted the strengths and weaknesses of different countries' manpower (see *Nature* **307**, 399; 1984). In the United Kingdom, the Science and Engineering Research Council (SERC) has been trying to ensure adequately trained manpower to support this emerging technology and has just published two reports prepared for it by the Institute of Manpower Studies (*Enabling Manpower for Biotechnology* and *The Biotechnology Brain Drain*).

There are now almost 2,000 professional specialist personnel active in novel biotechnology in the United Kingdom in addition to those engaged in traditional areas such as brewing. While a precise definition of a "biotechnologist" is inappropriate, most individuals being regarded as, say, microbiologists, biochemists or biochemical engineers, it is clear that, on the science side at least, few people would be regarded as professional biotechnologists without three or more years postgraduate experience; on the process engineering side, however, the entry threshold is more likely to be a first rather than a higher degree.

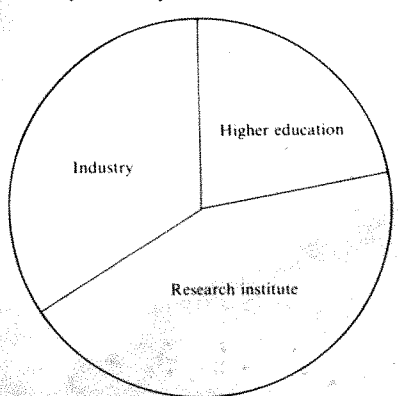


Fig.1 Distribution of biotechnologists in UK institutions.

The 2,000 specialists in the United Kingdom are employed in more than 100 organizations, some with just one or two, others with more than 50. One research laboratory employed over 100. The type of organization involved did not, however, indicate the actual employment level — two major pharmaceutical companies, for example, each employed fewer than 25 specialist biotechnologists, much of their basic research being contracted out to university-based research groups.

The commercial sector accounted for nearly half the employment at professional level, with research institutions taking about a third and the balance in higher education, although in the latter it was sometimes difficult to isolate applied biotechnologists from those focusing on more fundamental aspects of science (Fig.1). With the rapid growth of commercial companies spinning off from academic departments, consultancies and so on, the dividing line between the sectors is also becoming increasingly blurred.

With the exception of junior staff and chemical engineers, who were often recruited either after a first or possibly master's degree, most recruits to biotechnology had had some relevant postgraduate research experience and were typically in their mid to late 20s or early 30s. Apart from new companies or centres starting up, the numbers recruited rarely exceeded ten vacancies in a year in any one company. Wastage rates were also generally low. The only significant shortage of suitably experienced personnel was in fermentation technology, particularly microbial physiologists, biochemical engineers and bioscientists with research and development experience of plant and tissue cell culture. There was also a shortage, as in many other technologies, of people who could combine technical and managerial expertise.

One particular concern of the past few years has been the potential scale of the brain drain (see *Nature* **308**, 572; 1984). The study estimated that about 250 UK nationals had gone abroad since the mid-1970s, representing perhaps 13 per cent of the number currently employed in the United Kingdom. The peak flow was about 30 per annum in 1981 and 1982. UK nationals were found working in 13 countries; the main destinations were the United States (46 per cent), followed by Switzerland (16 per cent) and then Canada,

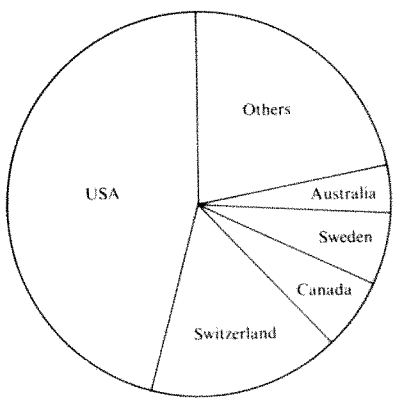


Fig.2 UK biotechnologists taking positions overseas.

Sweden and Australia (Fig.2). The largest loss was from higher education institutions and the main flow was to commercial organizations. More than 65 different overseas organizations were known to have recruited UK nationals. One recruited at least 16 UK nationals, four others took four or more each. One in three of those who had gone overseas had changed jobs since leaving the United Kingdom, and one in eight had also changed countries.

Typically the leavers were aged 26 to 30, although one in three was over 30. Nearly one in three went to "senior" jobs overseas, at professorial or departmental head level. They reported that the main reason for leaving was not salary but the lack of "suitable" opportunities and jobs in the United Kingdom. Most of them did not expect to return to the United Kingdom because they would be unwilling to take a reduced salary and standard of living. Thus while salary is not a motivation in leaving, it is a barrier to returning. While the flow overseas included a number of people who had left senior appointments in the United Kingdom and were unlikely to return, some were young postdocs moving overseas for experience, part of the normal international interchange of scientific personnel. While the outflow had no significant impact on individual organizations, such losses being only one part of their wastage to other employers, the flow was regarded as a lost opportunity and weakening of the United Kingdom's scientific base. The future flows, in either direction, will depend on job opportunities in the United Kingdom and salary differentials. □

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UNIVERSITY OF BIRMINGHAM

Faculty of Medicine and Dentistry

DEPARTMENT OF IMMUNOLOGY

Applications are invited for the post of Research Fellow 1A funded by Arthritis and Rheumatism Council for up to two years from 1 September 1984. Project will study the molecular specificity and clonality of rheumatoid factors and involve the production of monoclonal antibodies to human IgG epitopes and rheumatoid factor idiotopes.

Salary on scale £7,190 — £11,615 with superannuation maximum starting salary £8,975.

Further information Dr J Jefferis, Department of Immunology. Applications (three copies to Assistant Registrar, Medical School, Birmingham B15 2TJ by 28 June 1984) quote ref RF/IMM/NGA. (2881)A

THE UNIVERSITY OF MANCHESTER

TEMPORARY LECTURER IN PHYSICS

Applications are invited for the above post tenable from October 1st, 1984 for a period of one year. Applicants should have an interest in one of the current research areas of the Department, namely Astronomy, Radio/Astronomy, Low temperature and Condensed Matter Physics, Atomic, Molecular and Polymer Physics including Liquid Crystals, Optics, Nuclear Structure Physics, Particle Physics and Theoretical Physics.

Salary range £7,190 — £8,530 pa (Superannuation).

Applications with names of three referees should be sent to the Registrar, The University, Manchester M13 9PL by June 29th. Quote ref 140/84/N. (2372)A

UNIVERSITY OF GUELPH

COLLEGE OF BIOLOGICAL SCIENCE

PRE AND POST DOCTORAL POSITIONS

Available for training in the Molecular Biology of post transcriptional control with special emphasis on structure and function of mRNP complexes and bio-genesis of ribosome particles in procaryotic and eucaryotic systems. Studies of this laboratory are directed to examining molecular events which occur during changes in growth rate and differentiation. Teaching opportunities available. Stipends for post doctoral fellows are $17,500 to $21,000 and according to departmental guidelines for pre doctoral fellows.

Please send résumé and names of three referees to: Bruce H Sells, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1. (NW598)A

INSTITUTE OF CHILD HEALTH
UNIVERSITY OF LONDON

RESEARCH PSYCHOLOGIST

Psychologist/Human Biologist required for one year in the first instance, but with possibility of renewal, to undertake a feasibility study on the adverse physical and psychological effects of the intensive training of young athletes. The study is funded by the Sports Council and directed by Professor Philip Graham and Dr Peter Helms. The feasibility exercise may be followed by a major study. The successful applicant will have some previous research experience. Starting salary within the range £10,710 to £13,515 per annum plus London Weighting, according to qualifications and expericence.

Further details and application forms from the Assistant Secretary (N258804), Institute of Child Health, 30 Guildford Street, London WC1N 1EH, to whom completed application forms should be returned by 6 July 1984. (2304)A

Entomologist

Creative career opportunity for a Young Research Professional

We are one of the world's largest chemical companies and a major supplier of agricultural chemicals to U.K., Europe and the Middle East. Our research and development centre at King's Lynn has an international reputation for excellence and with our continued expansion we now wish to add a high calibre research Entomologist to our multi-disciplined team.

Ours is an intensely creative environment — originating and developing new products in pesticide science to the highest standards.

We would expect you to be in your twenties with at least a good honours degree in a biological discipline and a knowledge of agricultural entomology. You will need to be experienced in laboratory, glasshouse and small plot field trials and be able to carry out these with minimum supervision. You must have the research ability to progress ideas to a successful conclusion.

It has always been the policy of Dow as an international organisation to encourage responsibility at an early stage in your career with the Company. For someone who has the enthusiasm, flexibility and the creative input needed for successful research, the initial salary and benefits are excellent and future career prospects on an international scale, impressive.

To find out more please write or telephone for an application form to: Liz Sim, Personnel Officer, Dow Chemical Company Limited, Crossbank Road, King's Lynn, Norfolk PE30 2JD. Telephone: King's Lynn (0553) 61251.

DOW*

* Trade mark of The Dow Chemical Company (2897)A

AUSTRALIAN INSTITUTE OF MARINE SCIENCE

DIRECTOR

The Australian Institute of Marine Science is a Statutory Body established by Act of Parliament in 1972. The main functions of the Institute are:

- to carry out research in marine science;
- to arrange for the carrying out of research in marine science by any other institution or person;
- to co-operate with the other institutions and persons in carrying out research in marine science;
- to provide any other institution or person with facilities for carrying out research in marine science or otherwise assist any other institution or person in carrying out research in marine science;
- to collect and disseminate information relating to marine science, and in particular to publish reports, periodicals and other papers relating to marine science.

Under the general direction of a Council, the Institute is managed by a Director who holds office for a period not exceeding seven years, although he or she is eligible for re-appointment. The present Director will complete his seventh year in office on August 22, 1985, and expressions of interest in appointment to that position are now sought.

The Institute's headquarters are in a modern and well-equipped laboratory complex in pristine waterfront surroundings 55 km by road from the city of Townsville in North Queensland. The complex itself occupies a floor area of 10,000m² on several levels and incorporates a major library, computer facility, electronic and mechanical workshops and extensive laboratory space with comprehensive scientific instrumentation. In its position facing the Coral Sea, the Institute also has docking for its research vessels which include the 24.4 m R/V Lady Basten, the 13.66 m R/V Sirius, the 21 m R/V Harry Messel, on long term charter, and a number of smaller craft. A fully-equipped dive locker and recompression chamber service the needs of research underwater.

The present staff is 104 full time and 10 part time appointees including 20 research scientists. There are at present five multi-disciplinary programs addressed to coastal wetlands, nearshore physical processes and productivity, coral reef metabolism, coral reef ecology and the physical, chemical and biological processes associated with the shelf and adjacent seas, particularly but not entirely of the Great Barrier Reef. The major thrust of current research is in the environment of the Australian marine tropics.

Considerable emphasis is placed on interaction and collaboration with individuals and research organizations with complementary interests, both within Australia and overseas. The Institute has close and formal affiliations in research with James Cook University in Townsville. For interaction with collaborators further afield, the Institute maintains six fully self-contained cottages and six bachelor apartments, all on site. The accommodations also enable the Institute to host frequent workshops and symposia.

The Institute is fully committed to research. Although its functions do not include teaching, it regularly provides facilities for research by graduate students and it operates its own post-doctoral award scheme to augment in-house research activities.

Qualifications

The office of the Director is one of considerable seniority and responsibility. The successful appointee must have well-recognized academic qualifications and a demonstrable, and firmly established and active international reputation in some field or fields of marine science. Candidates must also have a broad grasp of marine research, and understanding of and enthusiasm for multidisciplinary activity and a depth of understanding and experience in research management in all its aspects including interaction with the agencies of Government. Enquiries in the first instance may be made by writing in confidence to the address given below. Formal applications should include documentation of professional experience and achievement (c.v.) and the names of not more than 5 referees. Applications must be dispatched no later than **September 1, 1984.**

Dr John L. Farrands, CB, FTS
Chairman
Australian Institute of Marine Science
P.M.B. No. 3
Townsville Mail Centre
Queensland 4810
Australia

(W1041)A

NIHE

NATIONAL INSTITUTE FOR HIGHER EDUCATION DUBLIN

Applications are invited for a:-

LECTURESHIP IN CHEMICAL SCIENCES

The School of Chemical Sciences is centrally involved in the BSc degree course in Analytical Science, in addition to servicing other courses, and possesses a comprehensive range of modern analytical instrumentation. The appointed candidate will become involved in the implementation and further development of teaching programmes and will be expected to initiate and develop programmes of research and to become involved in expanding links with industry.

Applicants should have interests in one or more of the following areas:

Analytical Science (instrumental aspects, inorganic analysis, separations science),

Applied Chemistry (areas of applied inorganic, organic or physical chemistry).

Applicants should be well-qualified academically with appropriate research, teaching or industrial experience.

Salary Scale: Lecturer IR£13,797 - IR£18,807
Assistant Lecturer IR£10,727 - IR£13,218

Application forms, and further details, are available from the Personnel Office, National Institute for Higher Education, Glasnevin, Dublin 9, Ireland. Closing date: 3 August 1984.

(2875)

OXFORD POLYTECHNIC
Chipsy Lane, Headington, Oxford OX3 0BP

DEPARTMENT OF BIOLOGY

Postgraduate Research Training

Three opportunities for research training, leading to MPhil/PhD (CNAA), will exist in the department from 1 September 1984. The following project areas are available:

Photoperiodism and Reproduction; Feedback control mechanisms on ion absorption and transport in higher plants; Allosteric models and haemoglobin; Production and characterisation of monoclonal antibodies for use in a diagnostic kit to detect demyelination; Biochemical mechanisms of resistance to aminoglycosides in clinically isolated bacteria; Laboratory selection for pesticide resistance in predatory mites; Glycoprotein metabolism in nerve terminals; *In vitro* construction of Tn5-derived transposons carrying novel selective markers and transactivated oriT regions; Physiological and biochemical indicators of alcohol consumption in support of clinical therapies; Numerical aspects of species diversity in insects.

Further details of the projects and of research in the Department available from the Postgraduate Tutor. Two of the posts are Researcher A appointments (£4,947 pa under review) associated with some teaching duties in physiology and biochemistry respectively.

Applicants should possess a good honours degree in an appropriate subject. They should provide a curriculum vitae including full details of any experience relevant to the project they nominate, and the names of two academic referees. Applications should be sent as soon as possible to:

Oxford Polytechnic, Chipsy Lane, Headington, Oxford OX3 0BP. Tel 0865 64777.

DEPARTMENT OF GEOLOGY AND PHYSICAL SCIENCES

Temporary Lecturer II/ Senior Lecturer in Geology

Ref. GPS/ 31

Applications are invited for a one-year, full time temporary post in the Department from suitably qualified candidates. The post will be available from 1 September 1984 to 31 August 1985.

The successful candidate will in part, be responsible for the teaching of Petrology and Mineralogy as part of the Polytechnic's modular degree course. Petrology and Mineralogy are taught within the named degree in Earth Sciences and the Geology single field honours degree.

Teaching responsibilities will be in at least two of the following areas; Metamorphic Petrology; Igneous Petrology with special reference to Plutonic Rocks; Introductory Mineralogy and Petrology. In addition the successful candidate will be required to supervise undergraduate field trips, may be required to teach geological map work and will be encouraged to participate in research.

Salary Scale LII £7,215 — £11,568
Salary Scale SL £10,683 — £13,443 (under review)

Further details and application forms are available from the Staffing Department, ext 364, Oxford Polytechnic, Chipsy Lane, Headington, Oxford OX3 0BP. Tel: 0865 64777.

The closing date for these posts is 22 June 1984.

(2890)A

UNIVERSITY OF LONDON INSTITUTE OF PSYCHIATRY

DEPARTMENT OF PSYCHOLOGY

Applications are invited for a

LECTURESHIP

The successful candidate will be required to provide a service to the Neurosurgical Unit and to the Epilepsy Ward of the Bethlem Royal and Maudsley Hospital. Applicants will also be expected to participate in the teaching of the Department on the MPhil Clinical Psychology Course and in the supervision of trainees. They must be capable of taking an initiative in developing the clinical and research services of psychologists in this new unit. Applicants should possess appropriate postgraduate qualification in clinical psychology and relevant experience is desirable.

Salary in accordance with the University of London Lecturer Scale £7,190 — £14,125 plus London Weighting allowance.

Application forms and fuller job description obtainable from Anne Stevens, Department of Psychology, De Crespigny Park, London SE5 8AF, and should be returned by Friday, 29th June 1984. (Tel: 703-5411 ext 321). (2303)A

Immuno-Diagnostics R&D

Our clients are the UK subsidiary of an innovative, and highly successful, international health care organisation. As part of its commitment to continued advancement, the UK diagnostics division is now undergoing a major expansion and seeks several experienced scientists within its R & D group.

The appointments will be based in new purpose-built premises in a pleasant part of the Southern Home Counties. They command an attractive remuneration package, including generous relocation expenses if required.

Immunoassay Specialists

2 PROJECT LEADERS, each taking responsibility for developing enzyme immunoassay techniques for a number of novel diagnostic kits.

Applicants should ideally hold a PhD and have several years' post-doctoral experience in immunoassay techniques, in conjunction with the use of enzymes. In addition they should have good written and verbal communication skills with the ability to direct and motivate staff.

5 PROJECT SCIENTISTS, each becoming a member of an enzyme immunoassay development team working with monoclonal antibodies.

Candidates should have at least a BSc in a relevant science and experience of immunoassay techniques, ideally also using enzymes.

Protein Chemist

To undertake the development of new enzyme monoclonal antibody reagents, and provide technical expertise and guidance for the improvement of existing products.

The ideal candidate would be a PhD protein chemist, with at least two years' relevant postdoctoral research experience, including the preparation of enzyme conjugates for immunodiagnostics.

Cell Culture Scientist

The main task will be to use tissue culture techniques to establish and maintain the production of monoclonal antibodies from hybridoma cells.

The successful candidate is likely to hold a PhD and have several years' post doctoral experience with project responsibility for tissue culture techniques.

Please write in confidence for an application form, or phone if you would like to discuss the appointments further:

Talentmark
Leaders in Health Care Recruitment



Bryan J. Oswald, Talentmark Limited,
King House, 5-11 Westbourne Grove,
London W2 4UA. Tel: 01-229 2266

(2363)A

SQUIBB

R&D DIRECTOR
Japan

Squibb, a major international pharmaceutical company, currently is seeking candidates for the position of R&D Director in its Japanese affiliate with offices in Tokyo. The Director must provide strong intellectual and administrative leadership to a staff of about 50 scientists who have responsibility for the registration in Japan of new human ethical and animal health products. In meeting these responsibilities, the department monitors Japanese regulatory requirements, oversees preclinical and clinical studies conducted in Japan, and prepare the necessary registration documents. Throughout this process, close liaison is maintained with the central research laboratories in the United States.

The successful candidate will have at least 15 years experience in the health care field after receiving an MD or PhD degree (or equivalent) and must be fluent in both Japanese and English.

Interested candidates should send their current resume directly to: **Mrs. Anne V. Corey, International Personnel Director, Squibb Corporation, P.O. Box 4000, Princeton, NJ 08540.** An equal opportunity employer.

(NW577)A

BATTELLE-KETTERING SENIOR POSITIONS

Battelle-C.F. Kettering Research Laboratory, located in Yellow Springs, Ohio, has two research positions available. The laboratory's research programs are oriented toward increasing agriculture productivity through research in the general areas of symbiosis, nitrogen fixation and photosynthesis.

One position requires a PhD in molecular genetics with related experience in appropriate bacterial and/or plant sciences. A PhD in plant sciences and five years experience is required for the second position which will serve to complement our current expertise and ongoing research activities. The successful applicants will be expected to support their research efforts by obtaining externally funded grants and contracts.

Battelle offers a competitive salary, excellent employee benefits, and career growth opportunities. Applicants with appropriate interests and expertise should send their résumé to Richard Shaw, Employment Department U-2.

Battelle

Memorial Institute
505 King Avenue • Columbus, OH 43201
An Equal Opportunity/Affirmative Action Employer

(NW593)A

THE AUSTRALIAN NATIONAL UNIVERSITY
JOHN CURTIN SCHOOL OF MEDICINE RESEARCH
Postdoctoral Fellow/ Research Fellow/ Senior Research Fellow (several positions)

Applicants for these research positions should have a PhD degree (or equivalent qualification) and expertise in any of the areas of research listed below. A number of appointments will be available from early 1985.

DEPARTMENT OF BIOCHEMISTRY: (Head: Professor F. Gibson)
Energy-transducing ATPase; membrane assembly; bioenergetics; mechanism of action of classical and multifunctional enzymes; molecular biology; gene expression; differentiation; oncogenesis.

DEPARTMENT OF EXPERIMENTAL PATHOLOGY: (Head: Professor P.C. Doherty)
Biology and biochemistry of tumour metastasis, malignancy and host response; viral and auto-immune pathological process in the central nervous system; vascular and lymphatic pathology; inflammation; pathogenesis of malaria.

DEPARTMENT OF HUMAN BIOLOGY: (Head: Dr R.L. Kirk)
Molecular biology of inherited disease, cytogenetics and somatic cell genetics; human population genetics, including DNA polymorphisms.

DEPARTMENT OF IMMUNOLOGY: (Head: Professor B. Morris)
Ontogeny of the immune response; immunology of the foetus and pregnancy; experimental surgery of the embryo and feotus; embryo transplantation; genetics of disease resistance; reproductive and developmental biology; tissue transplantation; physiology of the lymphatic system.

DEPARTMENT OF MEDICINE AND CLINICAL SCIENCE: (Head: Professor W.F. Doe)
Mucosal immunity and inflammation; neutral proteases; interleukins and differentiation of bone marrow cells, macrophages and natural killer cells; pathogenesis of inflammatory bowel disease and colon cancer; neurophysiology of intestinal motility; calcium and cell regulation; malignant hyperpyrexia; asthma; platelets, atheroma and haemostasis; transplantation biology.

DEPARTMENT OF MICROBIOLOGY: (Head: Professor G.L. Ada)
Properties of infectious agents, especially viruses; epidemilogical studies of, and immune responses, particularly cell-mediated responses, to viral infections; transformation of cells by viruses; engineering of viruses to make better vaccines; surveillance mechanisms against tumours; membrane structures and antigens, such as histocompatibility antigens, involved in cell-cell recognition.

DEPARTMENT OF PHARMACOLOGY: (Head: Professor D.R. Curtis)
Mammalian central transmitters; pain and analgesia; mammalian peripheral sympathetic synapses; biophysics of smooth muscle; nerve growth factors.

DEPARTMENT OF PHYSICAL BIOCHEMISTRY: (Head: Professor L.W. Nichol)
Biological macromolecules; interacting protein systems; multienzyme complexes; enzymes of sulphur metabolism; protein conformation and hydration; secretory and blood proteins.

DEPARTMENT OF PHYSIOLOGY: (Head: Professor P.W. Gage)
Synaptic transmission at peripheral and central synapses; effects of general anaesthetics; calcium ionic and gating currents; charge movements in nerve and muscle; contractile activation in muscle; visual information processing.

EXPERIMENTAL NEUROLOGY UNIT: (Director: Professor R. Porter)
Neurophysiology; spinal cord; synaptic transmission at central synapses; respiratory neurophysiology; somatosensory pathways; control of movement; physiology of the cerebral cortex in primates.

INFLUENZA VIRUS IMMUNOCHEMISTRY LABORATORY: (Dr W.G. Laver)
Structure and variation of influenza virus antigens and their antibodies.

CONDITIONS OF EMPLOYMENT: Salary will be in accordance with qualifications and experience within the range: Senior Research Fellow: $A35720 — $42607 p.a.; Research Fellow: $A25571 — $33594 p.a.; Postdoctoral Fellow Grade 1 (at fixed point on scale) $A22041 — $25249 p.a. ($A1 = £0.64p approx.).

Appointment as senior Research Fellow/Research Fellow will be for up to three years initially with the possibility of extension after review to maximum of five years; as Postdoctoral Fellow for one or two years initially with possibility of extension to maximum of three years.

Grants are provided towards travel and removal. Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. The University reserves the right not to make an appointment or to make an appointment by invitation at any time. Maternity leave is available.

Prospective applicants are encouraged to write to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or to the Acting Registrar Australian National University, GPO Box 4, Canberra, ACT2601, Australia, for further information before lodging applications with the Acting Registrar of the University. Closing date for receipt of applications is *17 August 1984.*

The University is an equal opportunity employer. (W1052)A

Agricultural and Food Research Council

SECRETARY TO THE COUNCIL

The present Secretary, Dr Ralph Riley, FRS, is retiring in mid-1985 and a successor is being sought.

The primary aims of the Council are to advance scientific knowledge relevant to agriculture, horticulture and food supply; to exploit this knowledge to increase the efficiency of the industries concerned; and to safeguard and improve the quality of food.

The Secretary is the Chief Executive of the Council. The main responsibilities of this position are:

☐ The overall management and scientific leadership of the Agricultural and Food Research Service which has an annual budget of about £100m and some 4250 staff. About half of the budget is provided by the Department of Education and Science from the Science Budget.

☐ Chief representative of the Council in its capacity as contractor to the Ministry of Agriculture, Fisheries and Food for commissioned research which accounts for the other half of the budget.

☐ All secretariat functions of the Council.

☐ Accounting Officer for the Council's grant-in-aid.

☐ A Member of the Advisory Board for the Research Councils.

Applications are sought from individuals with a distinguished background in a science or technology relevant to agriculture and/or food, who can also demonstrate that they possess the necessary management skills and judgment for this appointment.

It is likely that eligible candidates are today holding senior positions in research, either within industry or academia.

The age of the successful candidate must allow for at least five years in the post.

The present salary of the post is £35,125 p.a. A contribution towards relocation expenses may be made.

Those interested should write in confidence enclosing a brief curriculum vitae to the Management Consultants who are advising the Council on this recruitment, as follows:

The Chairman
Spencer Stuart Management Consultants
Brook House, 113 Park Lane
London W1Y 4HJ

(2305)A

THE MEDICAL COLLEGE OF ST. BARTHOLOMEW'S HOSPITAL

(University of London)
West Smithfield, London EC1A 7BE.

'NEW BLOOD' LECTURESHIP IN EPIDEMIOLOGY AND MEDICAL STATISTICS

Applications are invited from non-medically or medically qualified scientists experienced in biostatistics and epidemiology for the above 'New Blood' Lectureship tenable within the Department of Environmental and Preventive Medicine. This department is currently conducting case-control and prospective studies into the aetiology of disease and multi-centre clinical trials of disease prevention. The Lecturer would work as a member of an epidemiological research group and be expected to play an important role in the initiation of new research projects.

The appointment will be available from September/October 1984 and will be for a period of three years initially, with the possibility of renewal or subsequent transfer to a continuing appointment. Candidates should normally be aged under 35, and persons holding permanent university appointments in the UK are not eligible. The salary will be at an appropriate point on the relevant University Lecturer Scale.

Any applicants who would like to discuss the post are invited to contact Prof. N. Wald, Dept. of Environmental & Preventive Medicine at the Medical College, Charterhouse Square, London EC1M 6BQ (tel 01-253 0661 ext 148).

Applications in the form of a curriculum vitae (six copies please) should be sent to the Dean of the Medical College, West Smithfield, London EC1A 7BE (quoting ref. JR.14), together with the names of three referees, not later than 16th July 1984.

(2306)A

PROJECT MANAGER

TEN METER TELESCOPE

University of California

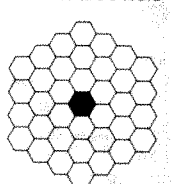
Applications are invited for the current opening of Project Manager (PM) for the University of California Ten Meter Telescope (UC TMT). The PM will have overall project responsibility for planning, engineering design, cost estimation, scheduling, and budget management for procurement, fabrication, and construction during the design and construction phases of the TMT and related facilities. In the determination of design and performance specifications, and the development of engineering design concepts, the PM will work in close cooperation with the Project Scientist.

Applicants should have demonstrated ability to complete major projects on time and within budget. Experience in the development of stand-alone research facilities is valuable. Knowledge of electro-mechanical and optical systems, computer environments, structural engineering, and laboratory research facility designs is desirable. Experience as manager of major research construction projects is a requirement. Applicants with degrees in engineering or physical sciences are preferred.

The PM will be an Associate Director of Lick Observatory reporting to a UC Executive Management Committee (EMC). Salary will be commensurate with experience and qualifications.

Applicants should send resume and the names of three persons who have been asked by the applicant to submit letters of professional reference directly to the Chair. All application materials should be received by September 15, 1984 and sent to:

Dr. Harold Ticho
TMT/EMC Chair
c/o Lick Observatory
University of California
Santa Cruz, California 95064



An EEO/AA Employer

(NW600)A

KING SAUD UNIVERSITY COLLEGE OF AGRICULTURE & VETERINARY MEDICINE QASSEEM, SAUDI ARABIA

Applications are invited on one year renewable contracts as:

1. ASSISTANT PROFESSOR (RESEARCH) — PHARMACOLOGY

Applicants must be Ph.D holders with experience in isolation, characterization and studies on therapeutic effects of isolated compound of plant origin.

2. ASSISTANT PROFESSOR RESEARCH — PHARMACOGNOSY

Applicants must be Ph.D holders with experience in isolation and characterization of medicinal principles in plants.

Applicants are expected to develop strong research programmes in the field of Medicinal Plants and Isolate Principles, and to characterize and study the mode of action in experimental animals.

3. TECHNICIANS IN PHARMACOLOGY AND PHARMACOGNOSY

Applicants must hold FIMLS or equivalent qualification and have substantial experience in advanced methods of chemical analyses with modern laboratory equipment, and experience in plant material extractions, chromatographies and methods of bioassoy.

Benefits include:

* competitive tax free salaries
* free furnished accommodation or housing allowance
* free yearly return air tickets for incumbent and family
* monthly transport allowance
* free medical/dental care
* children's educational allowance
* end of service gratuity

Applications should be sent, together with a photograph and non-returnable photocopies of academic credentials, to: Ms Carmel Donnachie, King Saud University Office, 29 Belgrave Square, London SW1.

Interviews will be held in London during the second week of July (approx.). (W1049)A

POSTDOCTORAL FELLOW

Postdoctoral Fellow interested in research in Cell Adhesion. Position for 2-3 years; starting salary: 18K.

Send CV and two letters of recommendation to: Fred Grinnell, Department of Cell Biology, The University of Texas Health Sci Ctr, Dallas, Texas 75235.

An Affirmative Action/Equal Opportunity Institution. (NW595)A

POSTDOCTORAL Position available to study the mechanism of coupling of muscarinic cholinergic receptors to the adenylate cyclase and phosphoinositide systems of the molecular basis of agonist induced desensitization of muscarinic cholinergic receptors in cultured cells. Send vita and three letters of reference to Dr T K Harden, Dept Pharmacol Univ North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC 27514. (NW596)A

UNIVERSITY OF EDINBURGH
DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH IN ORGANIC CHEMISTRY

Applications are invited for a Postdoctoral appointment to take part in a joint research programme investigating the synthesis of carbohydrates and analogues using cycloaddition reactions. The work will be carried out in association with Dr. R.M. Paton (University of Edinburgh), from whom further details may be obtained, and Dr. R.A.C. Rennie (Corporate Bioscience Group, ICI plc). The post is tenable for 3 years and is funded by the SERC Cooperative Awards Scheme. The salary will be on the RA/1A scale starting at £7630.

Previous experience in carbohydrate chemistry would be advantageous but is not essential.

Applications, giving curriculum vitae and the names of two referees, should be sent as soon as possible to Dr. R.M. Paton, Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh. EH9 3JJ.

Please quote reference no. 5176. (2337)A

UNIVERSITY OF SYDNEY

DEPARTMENT OF VETERINARY PHYSIOLOGY

(REFERENCE NO 22/01)

LECTURESHIP

Appointee will have research and teaching experience in mammalian physiology and an interest in contributing to development of self-instruction and audio visual programmes in courses of physiology offered in the Department to students of veterinary science and agricultural science. The major research interests in the Departments are physiology of the digestive and reproductive systems.

Salary range: $A24,353 — $31,994 pa (under review).

Appointments to lectureships are usually probationary for three years and the University reserves the right not to proceed with any appointment for financial or other reasons.

Applications, quoting reference no, including curriculum vitae, list of publications, the names of three referees to the Registrar, University of Sydney, NSW 2006, Australia, by 13 July 1984. Further general information available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (W1061)A

THE FLINDERS UNIVERSITY OF SOUTH AUSTRALIA

PROFESSOR OF CHEMISTRY
(Organic Chemistry)

Applications are invited from chemists working in any area of organic chemistry for appointment to a second Chair of Chemistry in the School of Physical Sciences. The Professors are expected to provide leadership both in research and in the teaching of chemistry in the University.

Currently, the major areas of research in organic chemistry are the synthesis of alicyclic compounds for NMR and substituent effect studies; organometallic chemistry, and the synthesis of aromatic compounds of medical interest. Other major areas of research in chemistry are the structure and reactivity of solids and solid surfaces, catalysis and electrochemistry.

Salary: $A51,006 a year (academic salaries are currently under review).

Enquiries of an academic nature may be directed to Professor B.G. Baker, School of Physical Sciences. Further information about the position, the conditions of appointment (including superannuation arrangements) and details required of applicants may be obtained from the Secretary General, The Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications should be lodged, in duplicate, with the Registrar, The Flinders University of South Australia, Bedford Park, South Australia 5042, by 17 August 1984. (W1058)A

UNIVERSITY OF WARWICK
POSTDOCTORAL RESEARCH FELLOW IN MICROBIOLOGY

In the Department of Biological Sciences to work on a project funded by the SERC. The work will be concerned with the modifications to mRNA Biochemistry in Cyanobacteria and their physiological implication. Salary will be on the Research 1A scale: £7,190 to £11,615 pa. The position is available from 1st July 1984 for two years three months.

Applicants should send a cv detailing relevant experience, together with the names and addresses of two referees, to the Executive Officer, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, quoting reference NGC/NAR, by 29th June, 1984.

Informal enquiries can be made direct to Dr N G Carr, Biochemistry Department, University of Liverpool, 051-709 6022 ext 2518. (2310)A

THE AUSTRALIAN NATIONAL UNIVERSITY
Research School of Biological Sciences

DEPARTMENT OF NEUROBIOLOGY

RESEARCH FELLOW/SENIOR RESEARCH FELLOW

Applicants should have experience in the analysis of insect central neural processing. Skill with microelectrode recording, dye filling, and the use of computers for data processing would be an advantage.

Salary will be in accordance with qualifications and experience within the ranges: Senior Research Fellow $A35,720 — $A42,607 pa; Research Fellow $A25,571 — $A33,594 pa ($A1: £0.64p: $US0.89 approx).

Appointment will be: Senior Research Fellow/Research Fellow normally up to three years with the possibility fo extension to maximum of five years.

Grants are provided towards travel and removal. Assistance toward housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should write to the Acting Registrar Australian National University GPO Box 4, Canberra, ACT 2601 Australia, or to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, for further particulars before submitting applications which close with the Acting Registrar of the University on 25 July 1984.

The University is an equal opportunity employer. (W1045)A

ADJUNCT ASSISTANT PROFESSOR

Position available January 1, 1985 for a molecular biologist with a primary interest in hormone action and gene expression. The applicant must have a PhD or equivalent, plus postdoctoral experience in techniques of molecular biology, including general recombinant DNA techniques, methods of chromatin structure studies and gene transfer. Applicants must be willing to work collaboratively with other molecular biologists in a research institute setting.

Send applications to Dr John Baxter, Chairman, Search Committee, Metabolic Research Unit, 671-HSE, University of California, San Francisco, CA94143. The University of California San Francisco is an Equal Opportunity/Affirmative Action employer. Women and minorities are encouraged to apply. (NW604)A

THE UNIVERSITY OF WESTERN AUSTRALIA
Perth

SENIOR TUTORSHIP IN GEOLOGY (PALAEONTOLOGY) (FIXED TERM)

Applications are invited from candidates with a higher degree and research experience in the field of palaeontology for a three year fixed term Senior Tutorship to commence in August 1984. All interested and qualified persons are encouraged to apply, but the department will particularly welcome enquiries from candidates with a background in the applications of palaeontology to sedimentology and stratigraphy. Some knowledge of micropalaeontology would also be an advantage.

The appointee will be asked to assist in the design and teaching of undergraduate courses in palaeontology and stratigraphy, including field training in sedimentary terrains. Supervision of honours students will also be required and the successful applicant will be encouraged to develop research programmes, either as an individual or in collaboration with others. Western Australian sedimentary basins comprise extensive areas and thicknesses of sedimentary rocks and almost all geological systems contain abundant fossil fauna. The Devonian, Permian, Jurassic, Cretaceous and Quaternary in particular provide material for significant research.

Current salary range: $A20991 - $A24047 per annum. Benefits include superannuation, fares to Perth for appointee and dependent family and removal allowance. Conditions of appointment will be specified in any offer of appointment which may be made as a result of this advertisement.

Applications in duplicate stating full personal particulars, qualifications and experience and the names and addresses of three referees, should reach the Staffing Officer, University of Western Australia, Nedlands 6009, Western Australia, by 28th July 1984. (W1057)A

UNIVERSITY OF NOTTINGHAM
MEDICAL SCHOOL
DEPARTMENT OF PATHOLOGY

WELLCOME TRUST LECTURESHIP

Applications are invited for the position of Lecturer (Medical or Non-Medical) in the Department of Pathology at Queen's Medical Centre, Nottingham. The purpose of this Lectureship is to enable a young medical or non-medical graduate to engage in a major research programme in some aspect of tissue pathology or related basic science subject. The Wellcome Trustees have made this award for three years in the first instance with the possibility of renewal for a further two years.

Applications should be supported by a 500 word summary outlining a proposed research programme. Prospective applicants should be less than 35 years of age and should not already hold a tenured post. Salary will be on the Lecturer scale with membership of USS.

Further information and application forms may be obtained from the Deputy Registrar, Medical School, Queen's Medical Centre, Nottingham NG7 2UH. (Telephone (0602) 700111 extension 3202), to whom completed applications should be returned by 10th July 1984. (2324)A

THE OPEN UNIVERSITY
Faculty of Science

TEMPORARY LECTURESHIP IN PHYSICS

Applications are invited for a temporary post as Lecturer in Physics at The Open University. The successful applicant will join a team of others to prepare course material for a second level physics course in Properties of Matters, using multimedia teaching techniques. In addition, the successful candidate is expected to carry out research. The Physics Discipline has individuals or groups active in the following areas: Atomic and Molecular Spectroscopy, Biomagnetism, Nuclear Structure Physics, Theoretical High Energy Physics, Solar Energy and Semi-Classical Approaches to Quantum Mechanics, and applications from candidates active in any of these fields would be especially welcome.

This post is available for three years from 1st September 1984. The salary will be at a point on the Lecturer A4 salary scale £7,190 — £14,125 pa depending on age and experience.

Candidates should hold a first degree and a PhD in Physics. An interest in distance teaching will be an advantage.

Application form and further particulars are available from The Assistant Secretary (Science) (4748/2), The Open University, Walton Hall, Milton Keynes MK7 6AA or telephone Milton Keynes (0908) 653481/653993: there is a 24 hour answering service on 653868.

Closing date for applications: 2nd July. (2368)A

CSIRO
AUSTRALIA

Chemical/Mechanical Engineer or Physicist

$A25,342 — $A37,274

DIVISION OF MINERAL ENGINEERING
CLAYTON VIC

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Mineral Engineering is a member of the Institute of Energy and Earth Resources.

FIELD: Mathematical Modelling.

GENERAL: The Division of Mineral Engineering, located at Clayton, in Melbourne, forms part of the Institute of Energy and Earth Resources. The Division conducts theoretical, experimental and application studies aimed at developing, improving and controlling industrial processes. Particular emphasis is placed on the treatment and handling of ores and mineral products.

DUTIES: In seeking its objectives, the Division undertakes extensive mathematical modelling of various operations in extractive metallurgy. Current activities include deterministic models of bubbles in fluidized beds, heat and mass transfer in various packed bed configurations, the sintering of ores, direct reduction of iron ores, etc. In all cases, a heavy emphasis is placed on proper validation of the models against experimental measurements.

The Division is expanding its modelling interests to include multi-phase flow systems with flow in 3 dimensions and concurrent chemical reactions, as found in various pyrometallurgical processes. The Division is seeking a chemical engineer, mechanical engineer or physicist with experience and interest in such numerical modelling. The appointee will undertake research on the development and validation of numerical models, initially of certain high temperature gas/slag/metal systems.

The Division is well equipped with computer systems and may, in addition purchase appropriate software packages for multi-dimensional flow computations.

QUALIFICATIONS: A PhD degree or equivalent qualifications plus research experience in an appropriate discipline, eg. Chemical Engineering, Metallurgy, Physics, together with experience relevant to the research to be undertaken.

Outstanding applicants may be considered for appointment at a higher classification.

TENURE: Indefinite with Australian Government superannuation benefits available.

APPLICATIONS: Quoting reference No A1537, should be directed to:

The Chief
CSIRO Division of Mineral Engineering
PO Box 312
CLAYTON VIC 3168
AUSTRALIA

By July 13, 1984.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W1043)A

CENTRAL BIRMINGHAM HEALTH AUTHORITY
THE CHILDREN'S HOSPITAL

SENIOR BIOCHEMIST/SCIENTIST

Applications are invited from suitably qualified graduates to work in the CELL CULTURE/ENZYMOLOGY section of the Department of Clinical Chemistry. This section is part of the laboratory which provides a Regional service for postnatal diagnosis of inherited metabolic disorders.

This is a specialised post and the work involves enzyme and metabolite assays in blood, fibroblasts and other tissues. Previous experience with cell culture techniques and enzymology, although not essential, will be an advantage.

Potential applicants are encouraged to visit the Department by prior arrangement with the Head of Department, Mrs Anne Green (021 454 4851 ext 290). Application forms/Job descriptions available from Personnel Officer, The Children's Hospital, Ladywood Middleway, Birmingham B16 8ET. Closing date for receipt of applications — 6th July 1984.

(2307)A

Senior Positions in Biochemistry and Nerve Cell Biology

The Sandoz Institute for Medical Research invites applications for the positions of Head of Laboratory (one in Biochemistry and one in Cell Biology & Cell Culture) tenable from 1 January 1985.

The Institute will be housed in well-equipped and spacious new laboratory accommodation which is being built within the precincts of University College London, with which strong academic links will be forged. The scientific staff will be up to 25–30 by 1987.

The Institute will embark on a programme of basic research in neurobiology, concentrating initially on mechanisms involved in nociception, with the long-term aim of developing new compounds of potential therapeutic value. Initially there will be six laboratory groups (Medicinal Chemistry, Biochemistry, Neuropharmacology, Cell Biology and Cell Culture, Immunology, Cellular Electrophysiology) which will collaborate on a range of individual projects.

Applicants for both positions need to have proven ability to organize and supervise the running of a research laboratory, and to contribute original ideas to a multidisciplinary research effort. Enthusiasm for interdisciplinary collaboration is essential.

Candidates for the Biochemistry position should have experience in the field of receptor-regulated mechanisms and/or the isolation and characterization of membrane proteins.

Candidates for the Cell Biology position should have general experience in cellular neurobiology and neuronal cell culture techniques, preferably with an interest in the biology of sensory neurons.

Salary negotiable, plus comprehensive benefit package including car. Assistance with relocation if required.

Applications by 16 July 1984, including cv and names of three referees, to Professor H.P. Rang, FRS, Director, Sandoz Institute for Medical Research, Department of Pharmacology, University College London, Gower Street, London WC1E 6BT (Telephone 01-388 5314), from whom further information can be obtained.

(2302)A

SANDOZ

Southampton THE UNIVERSITY

POST-DOCTORAL RESEARCH FELLOW
Department of Biology

Applications are invited from plant taxonomists to work with Dr F A Bisby and Dr R J White for three years commencing 1 October 1984. The collaborative project, supported by SERC and Biosis UK Ltd, involves experiments with disseminating taxonomic information from a computer database. The successful applicant will have a recent PhD and an interest in applying classical techniques in morphology, anatomy and nomenclature to the legume tribe Vicieae.

Starting salary £7,190 (under review) with annual increments and USS benefits.

Further information may be obtained if required from Dr R J White, Biology Department, Southampton University. Applications with curriculum vitae and names of two referees should be sent to Mr D A S Copland, Staffing Section, The University, Highfield, Southampton SO9 5NH as soon as possible, and not later than 20 July 1984. Please quote reference number 2052. (2323)A

THE UNIVERSITY OF WESTERN AUSTRALIA
PERTH
LECTURER IN ZOOLOGY (MAMMALIAN ECOLOGY) (FIXED FERM)

Applications are invited from suitably qualified persons for appointment for three years with some prospects of continuation to a Lectureship in the Department of Zoology. The Department has a tradition of marsupial studies in this appointment wishes to emphasize marsupial population biology. Exerpertise in mammalian population biology is sought; previous marsupial experience is not required. The Department runs a number of field stations suitable for the study of marsupial populations. The appointee will lecture to undergraduates generally and in a specialist unit, undertake research, and will be encouraged to lead graduate students.

Current salary range: $A24353-$A31994 per annum. Benefits include superannuation, fares to Perth for appointee and dependent family and removal allowance. Conditions of appointment will be specified in any offer of appointment which may be made as a result of this advertisement.

Applications in duplicate stating full personal particulars, qualifications and experience and the names and addresses of three referees, should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia 6009, by 21 July 1984. (W1059)A

UNIVERSITY OF LONDON
INSTITUTE OF PSYCHIATRY
The Bethlem Royal & The Maudsley Hospitals, London
LECTURESHIP IN OLD AGE PSYCHIATRY

Applications are invited for a second post of Clinical Lecturer in the newly established Section of Old Age Psychiatry. The applicant will be expected to initiate new research and teaching of the subject and to assist the Professor in administrative and clinical duties.

The post is open to suitably qualified psychiatrists or clinical psychologists. Psychiatrists should possess the MRC Psych or a higher postgraduate degree and would have an Honorary Senior Registrar contract. Clinical psychologists should possess an appropriate postgraduate qualification. A commitment to the social, psychological and scientific problems of old age is essential but a flexible view will be taken of what constitutes relevant experience, more emphasis being laid on academic track record than on formal training in the field.

Salary within the range appropriate form the discipline.

Applications should be in the form of a curriculum vitae (6 copies) with the names of two referees. They should be sent by 14th July to Professor Raymond Levy, Institute of Psychiatry, De Crespigny Park, London SE5 8AF from whom further particulars may be obtained.

(2364)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
POSTDOCTORAL RESEARCH FELLOW
DEPARTMENT OF PHYSIOLOGY

Required for 2 years for work on the electrophysiology and pharmacology of amino-acids in brain slices. A background in electrophysiology would be an advantage, though not necessarily using slices. Initial emoluments up to ££8,376 pa.

Application form and further details from the Establishment Officer (Ref: FP), St George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE. Closing date: 4th July. (2352)A

INSTITUTE OF CANCER RESEARCH
POST-DOCTORAL FELLOW

is required at the Institute's Chemical Carcinogenesis Section, Chester Beatty Laboratories, Fulham Road, SW3, for a project involving the investigation of the mechanisms by which polycyclic aromatic hydrocarbons are activated by metabolism in rodent and human cells and tissues. Appointment is for two years in the first instance. Salary in range £7,190-£8,975 pa, plus London Allowance of £1,186 pa.

Applications in duplicate with the names of two referees to the Personnel Officer, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref 300/G/50. (2354)A

TRANSLATORS, Russian to English, work at home in free time. Specialists in fisheries, aquatic biology and soil science reply to Scripta Technica (subsidiary to John Wiley & Sons, Inc. Publishers), 7961 Eastern Avenue, Silver Spring, MD, 20910. (NW597)A

UNIVERSITY OF ST ANDREWS
DEPARTMENT OF PHYSIOLOGY & PHARMACOLOGY
TEMPORARY LECTURER

Applications are invited for a Temporary Lectureship (three years) In addition to teaching duties, the successful candidate will be expected to carry out research within the field of "muscle energetics, metabolism and locomotion of Antarctic animals'. This work is supported by an award from the NERC Antarctic Research Special Topics Programme to Dr I A Johnston.

Some relevant postdoctoral or equivalent research experience in muscle research is essential. Excellent research facilities and support are available for studies of muscle physiology and biochemistry.

Salary at appropriate point within range £7,190 to £8,530 per annum (under reveiw), plus USS.

Further particulars are available from the Establishments Officer, The University, College Gate, St Andrews, Fife KY16 9AJ, to whom applications (two copies preferably in typescript) with the names of three referees, should be sent to arrive not later than 7 July 1984. (2891)A

KUWAIT
UNIVERSITY OF KUWAIT HEALTH SCIENCE CENTRE
FACULTY OF MEDICINE

Appointment in Anaesthesia
The Faculty wishes to appoint a Professor or an Associate Professor to lead the Division of Anaesthetics at the University Teaching Hospital (Mubarak Hospital). The successful applicant will have to be an established consultant, preferably at a University Undergraduate Teaching Hospital. The principal tasks will be to develop a high quality of anaesthetic service, and postgraduate teaching programmes and participate in undergraduate teaching programmes. Facilities for research have been set aside in the Department of Surgery and applications for research funds will be favourably considered.

The successful applicant will also be appointed as Head of the Clinical Department of Anaesthetics by the Ministry of Health and sit on the Discipline Committee for Anaesthesia for the State of Kuwait. *He may, in due course, be appointed Chairman.* The minimum duration of appointment will be 2 years.

Requirements for Appointment
Applicants for Professorship or for Associate Professorship should possess a PhD, or higher professional medical qualification (e.g. MRCP) in their respective speciality and have teaching experience and conducted and published research in their field. Associate Professors should have 9 years' experience past M.B., 4 as an Assistant Professor or its equivalent, i.e. Lecturer.

Conditions of Appointment
Salaries: Total monthly salaries will be within the following scales according to qualifications and experience (1 KD = £2.1, US$2.91 approx). Professor with clinical appointments: KD 1210-1370 (8 increments); Associate Professor with clinical appointments: KD 989-1149 (8 increments).

Clinical supplements: In addition to the above University salaries there will be a monthly clinical supplement paid by the Ministry of Public Health for ten months' a year to medical school staff with clinical service commitments. These are: Professor KD 200; Associate Professor KD 150.

Gratuity: There is a gratuity of one month's basic salary for each year employed payable on termination of contract.

Housing: Suitably furnished, air-conditioned accommodation, electricity and water are provided free of charge.

Medical Care: Free comprehensive treatment is available in Kuwait under the State Health Service.

Travel: Air tickets are provided from the country of recruitment for the appointee, spouse and up to three dependent children under 20 years, there after, return air tickets are issued annually to the country of citizenship or permanent residence. On termination of contract, air tickets are provided to the country of recruitment. A baggage and freight allowance is also provided. In addition, travel expenses for attending one scientific meeting a year are paid by the University up to KD 500 per year.

Vacation: Sixty days paid annual leave and various national holidays.

Education: This is normally provided free in State schools where the instruction is in Arabic. Staff who have to send their children to non-Arabic schools in Kuwait will have a part of the tuition fees of up to a maximum of three met by the University.

Taxation: There is no income tax in Kuwait. Currency is transferable without restriction.

Method of application:
Curriculum vitae in duplicate, which should include personal particulars qualifications with dates, career history, teaching experience, research accomplishments and clinical experience, should be sent together with the names of three referees to the Dean, Faculty of Medicine, University of Kuwait Health Science Centre, PO Box 24923, Safat Kuwait, to arrive no later than one month from the date of Advertisement.

Enquiries: Further particulars may be obtained from PROFESSOR M. D. VICKERS, Welsh National School of Medicine, Department Anaesthetics, Health Park, Cardiff, CF4 4XN, who is advising the Medical School on the appointment.

(W1051)A

THE AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH SCHOOL OF CHEMISTRY

POSTDOCTORAL RESEARCH POSITIONS

Applications are invited for postdoctoral research positions in the Research School of Chemistry. Current major research interests in the School are:

Organic Chemistry (Professor A L J Beckwith, Professor L N Mander, Mr R W Rickards, Dr J K MacLeod, Dr L Radom): including free radical reactions; mechanistic and synthetic organic chemistry; reactive intermediates; organic and bio-organic chemistry of bilogically active compunds; ICR and mass spectrometry; ESR spectroscopy; theoretical organic chemistry.

Inorganic Chemistry (Professor B G Hyde, Professor A M Sargeson, Dr M A Bennett, Dr G B Robertson, Dr S B Wild): including solid state inorganic chemistry; synthesis, structure and reaction mechanisms of transition metal complexes; biomimetic chemistry; organo-transition metal chemistry; X-ray crystallography; resolution and reactions of chiral arsines and phosphines, Physical and Theoretical Chemistry (Professor D P Craig, Dr J Ferguson, Dr R Bramley, Dr T R Welberry, Dr D J Evans): including molecular and crystal theory; spectroscopy and photochemistry; photophysics and magnetic resonance; X-ray diffraction and theoretical studies of disordered materials; liquid state chemical physics.

There are three levels of appointment, Postdoctoral Fellow, Research Fellow and Senior Research Fellow. The level appropriate for each appointee will be determined by the University and will depend on qualifications and experience. Appointment as Postdoctoral Fellow will be for one or two years; appointment as Research Fellow and as Senior Research Fellow will be for three years in the first instance. Salary will be within the ranges: Postdoctoral Fellow $A22,041 – 25,249 pa; Research Fellow $A25,571 – 33,594 pa; Senior Research Fellow $A35,720 – 42,607 pa. Current exchange ranges $A1: Stg 64p: $US 90c. Reasonable travel expenses are paid and assistance with housing is given for an appointee from outside Canberra. Superannuation benefits are available. Maternity leave is available.

Further particulars on the appointments may be obtained on request to the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF,or to the Acting Registrar, Australian National University, GPO Box 4, Canberra, ACT 2601, Australia. There is no application form. Applicants should supply to the Acting Registrar of the University by 24 August 1984 a curriculum vitae, list of publication, and statement of research interests together with two passport-sized photographs, the names and addresses of three academic referees and the probable date on which the Postdoctoral Fellowship, Research Fellowship or Senior Research Fellowship, if awarded, could be taken up.

The University is an equal opportunity employer. (W1056)A

THE MEDICAL COLLEGE OF ST BARTHOLOMEW'S HOSPITAL

RESEARCH ASSISTANT

Applications are invited for an MRC-funded research position which it is hoped will be available for commencement in October, 1984 for 3 years. The project, under the supervision of Dr P.J. Lowry and Dr G. Gillies in the Protein Hormone Unit, is to investigate the control of release of hypothalamic corticotropin releasing factors employing the techniques of hypothalamic neurone culture, isolated pituitary cell bioassay, radioimmunoassay, immunoradiometric assay and chromatography, including HPLC. Starting Salary (Range 1B) £6,310 + £1,186 London Allowance. Applicants should possess, or hope to obtain a good Honours degree in Pharmacology, Biochemistry, Physiology or some other relevant subject.

Applications may be made in writing, enclosing a Curriculum Vitae to The Secretary, St. Bartholomew's Hospital Medical College, West Smithfield, London EC1A 7BE, quoting reference RES.16.

Closing Date: 28th June 1984. (2355)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF RADIATION ONCOLOGY

Applications are invited for the post of

UNIVERSITY RESEARCH ASSISTANT/SENIOR RESEARCH ASSISTANT/RESEARCH ASSOCIATE

in the recently formed Department of Radiation Oncology. The person appointed will assist in the development of human monoclonal antibodies and will be required to operate the Departments FACS 420 machine. Preference will be given to candidates with previous experience of flow cytofluorimetry and cell sorting.

Salary on the scale £6,310 – £11,615 per annum.

Applications, together with the names of three referees, should be received not later than 22 June, 1984, by the Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/537. (2308)A

UNIVERSITY OF OXFORD

NUFFIELD DEPARTMENT OF CLINICAL BIOCHEMISTRY

TEMPORARY RESEARCH TECHNICIAN

A short-term vacancy has arisen for a technician (M.L.S.O. Grade) to assist with studies on the regulation of insulin secretion. Experience of tissue culture would be an advantage. The appointment is for six months; however, the post may lead to re-appointment.

Applications including curriculum vitae and names of two referees should be sent to Dr S.J.H. Ashcroft, Department of Clinical Biochemistry, John Radcliffe Hospital, Headington, Oxford OX3 9DU, (Telephone: 0865 817377), from which further details may be obtained. (2345)A

Please mention

nature

when replying to these advertisements

YOU'VE GOT A BRIGHT IDEA

for new biological or chemical compounds.

WE HAVE THE CAPITAL

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We are not a pharmaceutical firm but a Swiss-based, independent financial group which is prepared to let you participate in a capital venture for the exploitation of your project.

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To obtain all the information required for initial selection, please write in English, French or German to:
DEBIOPHARM S.A. Petit-Chêne 38 - 1003 Lausanne
SWITZERLAND (W732)A

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT IN PROTEIN CRYSTALLOGRAPHY

Applications are invited for the above M.R.C. funded post for work on the three-dimensional structures of glutamate dehydrogenase, and a bacterial toxin. The structural work is being carried out in parallel with enzymological and protein chemical studies, and will be aided by use of advanced colour graphics and a powerful computer. Applicants should have experience in crystallography, preferably protein crystallography. Experience in diffractometer or oscillation camera data collection an advantage. Tenable for a fixed term from 1 October 1984 to 31 August 1986, with possibility of extension. Initial salary £7190 – £11160 a year on Range 1A. Applications (with c.v. and names of two referees) to Dr D W Rice or Professor P M Harrison, the University, Sheffield S10 2TN as soon as possible and not later than 5 July 1984. Quote ref: R106/G (2886)A

UNIVERSITY OF NEWCASTLE UPON TYNE

THE MEDICAL SCHOOL

DEPARTMENT OF PHYSIOLOGICAL SCIENCES

APPOINTMENT OF DEMONSTRATOR

Applications are invited for a post o Demonstrator (Medical or Non Medical) in the Department o Physiological Sciences. The appoint ment will be for up to three year commencing on or after 1st Octobe 1984. Applicants who do not have medical degree should either have PhD degree or be confidentl expected to have a PhD by 31s December 1984.

Commencing salary will be accorc ing to age, qualifications and exper ence on the Non-Medical Demor strators' scale £6,310-£8,530 pe annum, or for medically qualifie candidates on the Pre-Clinica Demonstrators' scale £7,170-£8,97 per annum.

Further particulars may b obtained from the Senior Admin strative Assistant, The Medica School, The University, Quee Victoria Road, Newcastle upon Tyn NE1 7RU, with whom application (three copies) together with th names and addresses of two referee should be lodged not later than 6t July 1984. (2339)A

Canadian Institute for Theoretical Astrophysics/ Institut Canadien D'Astrophysique Theorique

University of Toronto

DIRECTOR

The University of Toronto is seeking to appoint a tenured full professor of theoretical astrophysics. The successful candidate will be a scientist of established stature who must also possess both vision and administrative ability. In addition to his own research activities, his primary responsibility, at least initially, will be to ensure the growth of the institute, CITA, in substance and concept by providing scientific leadership and administrative guidance in collaboration with the national Council of the institute. The professorship will be in CITA, within the School of Graduate Studies, with cross-appointments foreseen in the Department of Astronomy and/or Physics. Send letter, CV and arrange for letters from three persons commencing on suitability for the described position to reach: Chairman, CITA Council, Canadian Institute for Theoretical Astrophysics, University of Toronto, Toronto, Ontario M5S 1A7 at the earliest possible time, and not later than 1 September 1984. In accordance with the regulations of Canadian immigration, preference will be given to Canadian citizens and permanent residents.

(NW605)

HILL FARMING RESEARCH ORGANISATION

Scientific Officer

Applications are invited for the post of Scientific Officer in our Systems Studies programme at Hartwood Research Station.

The Hill Farming Research Organisation is an Equal Opportunities Employer.

The Principal duty of the post will be responsibility for the day to day management of sheep systems studies at Hartwood. The successful candidate will be directly responsible to the project leader and will be expected to collect and record the necessary data.

Qualifications: A degree in Biology or Agricultural Science.

Salary: According to qualifications within the scale £5,682 to £7,765.

There is a non-contributory terminal salary superannuation scheme. Male members of staff must pay 1½% of salary in respect of widows' pension rights.

22 days annual leave

Application forms and further information may be obtained from the Assistant Secretary, Hill Farming Research Organisation, Bush Estate, Penicuik, Midlothian EH26 0PY to whom they should be returned not later than 29 June 1984.

(2348)A

WESTMINSTER MEDICAL SCHOOL
HISTOPATHOLOGY DEPARTMENT

Applications are invited for a

RESEARCH POST

which is based at the Vincent Square Research Laboratory and funded for two years. The research project is to evaluate immunocytochemical markers in soft tissue tumours.

Experience in histopathology techniques is essential, whilst a knowledge of immunocytochemical techniques, although not essential, would be useful. Salary on the MLSO scale with conditions of service comparable to Whitely Council.

Further information may be obtained by telephoning Dr Mary Leader on 01-828 9811 ext 2301. Curriculum vitaes should be sent together with the names and addresses of two referees to Dr Mary Leader, Histopathology Department, Westminster Medical School, 17 Horseferry Road, London SW1P 2AR.

Closing date for applications: June 30th. (2359)A

THE UNIVERSITY COLLEGE OF WALES ABERYSTWYTH
POSTDOCTORAL RESEARCH: MICROBIAL PHYSIOLOGIST

Applications are invited for a Postdoctoral post funded by the SERC Biotechnology Directorate, and concerned with biotransformations by species of Clostridium. The applicant will join an established research group led by Professor J G Morris, studying fermentation processes in anaerobic bacteria maintained in batch and continuous flow culture. Informal enquiries may be made of Dr Douglas Kell by telephoning (0970) 3111, Ext 3055.

The appointment will be for up to 3 years, depending on the starting date, and will commence not later than 1 October 1984. The salary scale is £7190 to £11615 per annum (under review).

Application forms and further particulars may be obtained from the Staffing Officer, The University College of Wales, Old College, King Street, Aberystwyth, SY23 2AX (Tel (0970) 3177, Ext 207). Closing date for applications: 2 July 1984.(2336)A

Murdoch University

PERTH
WESTERN AUSTRALIA

PROFESSOR OF CLINICAL PATHOLOGY SCHOOL OF VETERINARY STUDIES

Applications are invited for the Chair in Clinical Pathology in the Division of Applied Veterinary Medicine. The holder of the Chair will be expected to have a sound knowledge of and experience in interpretive laboratory medicine, to facilitate the interface between Pathologists and Clinicians, to provide leadership in the teaching and service work of Clinical Pathology and in research activities in the Clinical areas of the School.

A statement about the University, the School and the responsibilities of this post, together with a copy of the conditions of appointment, are available on request from either the University or the Association of Commonwealth Universities.

SALARY
$A51,006 per annum.

Conditions of appointment include superannuation, long service leave, access to outside studies programmes, payment of fares to Perth for appointee and dependent family, removal and settling-in allowances.

PROCEDURE FOR APPLICATION
There is no prescribed application form. Applicants are invited to submit a full curriculum vitae supported by an appropriate letter including the names and addresses of three referees. Applications and requests for information should be sent to:

The Personnel Officer,
Murdoch University,
Murdoch, Western Australia, 6150

Applicants resident in the United Kingdom, Europe or Africa at the time of application should also forward one further copy to:

(W1050)A

The Secretary General,
Association of Commonwealth Universities,
36 Gordon Square,
London WC1H OPF, United Kingdom

*Applications will close on **31st August 1984.***

THE KENNEDY INSTITUTE
CELL BIOLOGIST

Post-doctoral position available to investigate the molecular basis of cell-to-cell interaction and lysosomal enzyme transfer. Interest/experience of sub-cellular fractionation and membrane biochemistry would be advantageous.

Salary on the Universities Research and Analogous Staff Scales depending on age. London Weighting paid in addition.

Applications with curriculum vitae and names of two referees to The General Secretary, The Mathilda and Terence Kennedy Institute of Rheumatology, Bute Gardens, London W6 7DW. (2313)A

HILLINGDON HEALTH AUTHORITY
Harefield Hospital
Harefield, Middlesex

CELLULAR IMMUNOLOGIST
to work in Transplant Unit

Recently qualified PhD or experienced graduate to work on human T cells, experience in cell culture and T cell cloning would be an advantage.

Salary appropriate to age and qualifications.

Curriculum vitae with names of two referees to Personnel Office, at the above address.

Closing date: 29th June 1984.

(2365)A

Public Service Commission of Canada | Commission de la Fonction publique du Canada

Research Scientist, Earth Structure Studies

Energy, Mines and Resources
Earth Physics Branch
Ottawa, Ontario

We require a geophysicist/geologist to conduct field investigations and research studies into the nature and structure of major geological features throughout the Canadian landmass. Emphasis will be on the application of deep seismic reflection techniques, including vibrosesis, and regional three-dimensional seismic refraction methods. Other geological and geophysical methods will be incorporated as necessary. Field investigations will be carried out in all parts of Canada, from the east and west coasts to the Arctic Ocean Basin. Research studies will demand extensive use of state-of-the-art computer-based processing and international methods.

You require graduation with a doctorate degree from a recognized university in geophysics, geology, physics or a related field, or a lesser degree with evidence of research experience and productivity equivalent to that of a doctorate degree. Extensive experience in the processing and interpretation of multifold seismic reflection data is also required. Knowledge of either English or French is essential.

We offer a salary ranging from $26,834 to $49,913 commensurate with your qualifications and experience. Effective 23 June 1984, the revised salary range will be $28,176 to $52,409.

Forward your application form and/or résumé, quoting reference number S-84-31-5523-JG47(G26), to:
Joan Girling (613) 593-5331
Public Service Commission of Canada
Ottawa, Ontario K1A 0M7

Closing date: 31 August 1984 (NW599)A

The Public Service of Canada is an equal opportunity employer Canada

UNIVERSITY OF BRISTOL

DEPARTMENT OF BOTANY AND ZOOLOGY

POST-DOCTORAL RESEARCH ASSISTANT

To investigate the microstructure of algal communities on estuarine mud surfaces, and to examine the effects of grazing snails on these communities and on surface stability. The research will utilize scanning electron microscopy and will involve the development techniques of frozen-hydrated specimen preparation. Research will be undertaken in collaboration with Dr R M Crawford and Dr C Little.

Experience in electron microscopy and some knowledge of microalgae is essential. Salary will be on the 1A salary scale. The appointment will be for two years starting on 1 October 1984.

Applications (citing 2 referees) to Dr R M Crawford, Department of Botany, University of Bristol, Bristol BS8 1UG by 1 July 1984. (2884)A

University of California Lawrence Berkeley Laboratory Announces an opening 1 January 1985 for Associate Laboratory Director and Head of Nuclear Science Division

The Lawrence Berkeley Laboratory is a multi-purpose research laboratory funded by the Department of Energy. Its Nuclear Science Division conducts a multi-million dollar research program in low, medium and high-energy nuclear physics. The Head of the Division has primary responsibility for the leadership and management of this program under the general direction of the Deputy Director for General Sciences and the Laboratory Director. As an Associate Director of the Laboratory, the incumbent will also participate in formulating the research policy and long-term direction of the Laboratory as a whole. Candidates must have had significant experience as practicing nuclear physicists and be of the general calibre of persons holding senior tenure positions in major research institutions. The successful applicant would be expected to serve as Associate Director and Division Head for an initial term of 3 to 5 years, subject to renewal for further terms by mutual agreement.

The Laboratory is seeking a respected and energetic nuclear physicist willing to play a strong leadership role at a challenging time. Qualifications must be such that it will be natural to consider him or her for a tenured appointment in the Department of Physics on the Berkeley campus of the University of California, as well as at the Laboratory. Interested applicants should submit a curriculum vitae, together with the names of three references to: Dr J.D. Jackson, Chairman, Nuclear Science Search Committee, c/o Employment Office, LAWRENCE BERKELEY LABORATORY, 1 Cyclotron Road, Berkeley, CA94720. Refer to Job A/2947. An Equal Opportunity Employer M/F/H. Deadline for applications: August 31, 1984. (NW594)A

LAWRENCE BERKELEY LABORATORY

KINGDOM OF SAUDI ARABIA
KING SAUD UNIVERSITY
COLLEGE OF AGRICULTURE
RIYADH, SAUDI ARABIA

Qualified personnel are invited to apply for the following posts of FACULTY MEMBERS (Professors, Associate Professors, Assistant Professors), LECTURERS, DEMONSTRATORS, RESEARCH ASSISTANTS and TECHNICIANS for the academic year 1984 -1985.

Department	Specialization	Qualification
I. Staff members:		
(1) Food Science	a. Human nutrition metabolism. b. Dairy technology. c. Food analysis.	Ph.D., Teaching experience, Arabic speaking.
(2) Animal Science	a. Poultry nutrition.	-do-
(3) Plant Production	a. Cytogenetics.	-do-
(4) Ag. Economics & Rural Sociology	a. Farm management. b. Marketing of agricultural products.	Ph.D., Teaching experience, Associate or Professors only, Arabic speaking.
(5) Home Economics & Nutrition (Female only)	a. Textiles and costumes.	Ph.D., Teaching experience, Arabic speaking.
II. Lecturers:		
(1) Home Economics and Nutrition (Female only)	a. Plant production (ornamentals) b. Plant production (field crops).	M.Sc., Teaching experience, Arabic speaking.
(2) Plant Production	a. Plant production (vegetable crops).	-do-
(3) Animal Production	a. Animal production	-do-
III. Demonstrators:		
(1) Food Science	a. Fish and meat technology. b. Food microbiology. c. Food analysis.	B.Sc. "B" average or higher, past experience in teaching, research is preferred, Arabic speaking.
(2) Plant Production	a. Horticulture (vegetable crops).	-do-
(3) Agricultural Engineering	a. Agric. Engineering.	-do-
IV. Research Assistants:		
	a. Food microbiology. b. Food chemistry and analysis. c. Horticulture (Pomology). d. Field Crops (Breeding).	Preferably M.Sc. with research experience in the field of specialization.
V. Technicians:		
	a. Human nutrition. b. Cereal technology. c. Analytical chemistry in the field of food science.	Experience in field of work, conducting and operating laboratory equipment. Experience and training in operating food and nutrition analyses equipment preferably IR, AAA, AA, GLC, HPLC. Good knowledge of English language.

Please apply to:
Dean, College of Agriculture,
King Saud University,
P.O. Box 2460,
Riyadh 11451,
Kingdom of Saudi Arabia.

(W1042)A

CSIRO

AUSTRALIA

Molecular Biologist

$A25,342 — $A37,274

DIVISION OF MOLECULAR BIOLOGY
NORTH RYDE NSW

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Molecular Biology is a member of the Institute of Animal and Food Sciences.

GENERAL: The Division, with a staff of nearly 100, has research programs in Animal Cell Growth and Development, DNA Maintenance and Modification and Gene Technology.

DUTIES: To conduct and direct ongoing research in the area of gene cloning and expression, with particular emphasis on the molecular genetic manipulation of prokaryotic and eukaryotic micro-organisms for the purpose of metabolic modification or the production of vaccine antigens (and other polypeptides of interest).

QUALIFICATIONS: A PhD degree or equivalent qualifications in biochemistry or a related discipline and preferably with relevant post-doctoral training. Broad experience in the various facets of molecular biology at the nucleic acid, protein and immunological levels would be an advantage.

TENURE: Indefinite with Australian Government superannuation benefits available.

APPLICATIONS: Stating full personal and professional details, the names of at least two referees, and quoting reference No A3782, should be directed to:

The Chief
CSIRO Division of Molecular Biology
PO Box 184
NORTH RYDE NSW 2113
AUSTRALIA

By July 13, 1984.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W1044)A

Structural Cellular/ Molecular Biologist

Small-Angle Neutron Scattering

The University of Missouri-Columbia, Division of Biological Sciences invites applications for a tenure-track position in the area of structural cellular and molecular biology at the assistant or associate professor level, dependent on qualifications. The position requires undergraduate and graduate teaching in cellular and molecular biology and a biological research emphasis that relies heavily on small-angle neutron scattering. Applicants must have had extensive experience, and demonstrated excellence in small-angle neutron scattering. The small-angle neutron scattering spectrometer at the University of Missouri Research Reactor in Columbia is one of only a few such instruments in the U.S. and is the highest flux steady-state reactor at any American University. The successful candidate will interact with a strong University faculty with interest in biological structures: nucleic acids, membranes, proteins, protein-nucleic acid complexes, and organelles. Modern biophysical instrumentation already on the Columbia campus include: NMR (300, 200 and 90MHz); scanning and transmission electron microscopes; mass, fluorescence, atomic absorption, and small-angle x-ray scattering spectrometers.

Applicants should submit by, August 20, 1984, curriculum vitae, statement of research and teaching interest, and names of three references to: Paul F. Agris, Chairman, Search Committee, University of Missouri, Biological Sciences, Tucker Hall, Columbia, MO 65211, 314-882-6426.

An Affirmative Action/Equal Opportunity Employer.

(NW602)A

Canadian Institute for Theoretical Astrophysics/ Institut Canadien D'Astrophysique Theorique

University of Toronto

SHORT TERM FELLOWS AND VISITING APPOINTMENTS

The community of theoretical astrophysicists in Canada, in association with the University of Toronto and with funding from the Natural Sciences and Engineering Research Council, has recently established a national centre for theoretical astrophysics. Pending appointment of a Director, after which longer term appointments will be considered, applications are invited for short term fellowships. These are intended to encompass visiting appointments at any level of seniority, and will be for a mutually convenient duration of up to one year, beginning immediately. Also included are postdoctoral and research associate positions, for a one year term with the possibility for renewal; these are likely to begin in the fall of 1984. Although candidates with interests spanning the whole spectrum of theoretical astrophysics are encouraged to apply, the Council of the institute will be attempting to concentrate interests at any given time to encourage interactions between Canadian and foreign investigators. Send letter, CV and arrange for three letters of recommendation to be sent to: Chairman, CITA Council, Canadian Institute for Theoretical Astrophysics, University of Toronto, Toronto, Ontario M5S 1A7 at the earliest possible time. Initial contact by letter or telephone (416-978-4165/6840) is encouraged for established scientists contemplating visits. In accordance with immigration regulations, certain portions of this advertisement are directed in the first instance to Canadian citizens or permanent residents. (NW606)A

Chair and Professor, Division of Nematology

Nominations and applications are invited for an 11-month, tenured position at the University of California at Davis to serve as Chair of the Division of Nematology. The Chair will also have some research, teaching, and student advising responsibilities. The usual appointment as Chair is for five to seven years, subject to renewal on review. Upon completion of service as Chair of the Division, the faculty member may return to full-time teaching and research. The position is available September 1, 1984.

Qualifications include a Ph.D. with experience in Nematology or closely related discipline, proven leadership and management qualities, and a record of scholarly and academic achievement.

Applicants should submit a *curriculum* vitae with a bibliography and reprints of pertinent publications, statement of research, teaching, and administrative interests and background in each, and the names and addresses of at least three references to: Dr. R. K. Webster, Acting Associate Dean and Search Committee Chair, College of Agricultural and Environmental Sciences, Dean's Office, 228 Mark Hall, University of California, Davis, CA 95616 USA. Applications should be received by August 1, 1984. Applications received later will not be considered unless the position is reopened for recruitment.

The University of California is an affirmative action/equal opportunity employer. (NW603)A

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF CHEMISTRY

RESEARCH ASSOCIATE

Applications are invited for the above NERC funded post to work with Dr J A Kerr on Kinetic and Mechanistic Studies of the photo-oxidation of Aldehydes under Simulated Atmospheric Conditions. Part of a research programme aimed at obtaining a better understanding of the chemistry of the lower atmosphere.

There will be an opportunity to register for a higher degree.

The post is tenable for up to 3 years from 1.10.84. Salary on the Research Associate 1B scale £6310 — £8530 plus superannuation. Starting point near the bottom of the scale.

For further particulars phone 021-472 1301 Ext 2559 quoting reference F3.

No formal application form. Three copies of application, including full Curriculum Vitae and naming three referees to Assistant Registrar (Science and Engineering), P O Box 363, Birmingham, B15 2TT by 29 June 1984. (2370)A

Southampton THE UNIVERSITY

NEW BLOOD LECTURESHIP IN GEOLOGY
(Re-advertisement)

Applications are invited for the post of Lecturer in Geology with special emphasis on the geochemistry of fluid-rock interactions as applied to metals and/or hydrocarbons. Applicants with interests in fields such as hydrothermal alteration, petroleum generation and migration, diagenesis, pore fluids, fluid inclusions or stable isotopes will be particularly welcome. This new blood post is intended to reinforce the research interests of the Department in applied geology. Salary scale: £7,190 × £450 (15) — £14,125 per annum (under review). The initial salary will depend on qualifications and experience. Further particulars may be obtained from Mr D. A. S. Copland, The University, Southampton SO9 5NH to whom applications (7 copies from United Kingdom applicants) should be sent not later than 2 July 1984, quoting reference No: 2039.

RESEARCH ASSOCIATE

To participate in formulation and solution of mathematical, statistical, and computer science problems in the analysis of macromolecular sequences. Candidates should have expertise in nucleic acid and protein sequences, the genetic code, probability, statistics, optimization theory and computer programming, and a master degree in mathematics. Salary $16,000 per year. Job site: Los Angeles area educational institution. Send Ad and curriculum vitae to Job #4126, PO Box 865, Sacramento, CA 95804 not later than June 29, 1984. (NW601)A

UNIVERSITY OF OXFORD

SIR WILLIAM DUNN SCHOOL OF PATHOLOGY
South Park Road,
Oxford OX1 3RE

MOLECULAR BIOLOGY OF MACROPHAGES

POST DOCTORAL RESEARCH
position Grade 1A
Salary: £7,190 — £11,615

A talented, well trained Molecular Biochemist is needed to initiate research into gene structure and expression in Macrophages. My group at the Sir William Dunn School of Pathology is supported by an MRC programme grant and works on macrophages secretion products, plasma membrane differentiation antigens and receptors.

The appointment, starting as soon as possible, is likely to be for at least four years.

Applications enclosing curriculum vitae and the names and addresses of two referees should be sent to Dr S Gordon, Sir William Dunn, School of Pathology, South Park Road, Oxford OX1 3RE. (2878)A

A challenging career in Diagnostic Reagents

LABORATORY HEAD

c.£15,000: Dartford, Kent

We are seeking to consolidate our activities in the field of Diagnostic Reagents by establishing a new laboratory within our Central Analytical Division at Dartford.

Managing a small team of experienced staff, you will be responsible to senior management for ensuring that the laboratory meets its major objectives, which include:

- Developing and introducing reliable quality control methods and procedures
- Establishing quality control standards and specifications
- Assessing the stability of products
- Assembling data for product registration
- Conducting technical audits
- Handling customer complaints

Qualified to honours degree level in a biological science, you should have several years' post-graduate experience in a range of applied techniques ideally involving diagnostic reagents. Experience of analytical development and quality assurance together with the ability to demonstrate good man-management and communication skills are also essential requirements.

The Wellcome Foundation Limited is a major group of pharmaceutical and chemical companies with headquarters in the UK and a turnover close to £700m.

Excellent benefits include five weeks' holiday, first class sports and social facilities and generous assistance with relocation expenses where appropriate.

Please write or telephone for an application form to Steve Menear, Personnel Officer, The Wellcome Foundation Limited, Temple Hill, Dartford, Kent. Tel. Dartford (0322) 23488, ext 2025. (2366)A

Wellcome

UNIVERSITY OF LONDON
ST GEORGE'S HOSPITAL MEDICAL SCHOOL

DEPARTMENT OF PHARMACOLOGY AND CLINICAL PHARMACOLOGY

MRC POST DOCTORAL POSITION IN CYCLIC NUCLEOTIDE RESEARCH

A 3-year MRC funded position (Ref TC 14) is available to work with Dr A C Dolphin on biochemical and/or electrophysiological aspects of the role of cyclic nucleotides in modulation of neuronal cell function. Immunocytochemical techniques will also be used. Experience in tissue culture would be an advantage, as it is envisaged that neuronal cells in culture will be used for much of the work.

The salary will be on the 1a scale, £7190: £7630: £8080 plus £1186 London allowance.

Applications, including curriculum vitae and the names of two referees should be sent to Dr A C Dolphin, Department of Pharmacology and Clinical Pharmacology, St George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE.

Further details can be obtained by writing to Dr Dolphin or by telephoning (01-672 1255 Ext. 4688). (2888)A

Head of Literature/ Information Services

A unique Career opportunity

Merck Sharp & Dohme Research Laboratories are establishing a Neuroscience Research Centre on a greenfield site near Harlow, Essex. The Centre will be one of the world's largest laboratories dedicated to Neuroscience research encompassing Medicinal Chemistry, Biochemistry and Pharmacology.

We are seeking to recruit an exceptional individual who will have the task of initially establishing, and ultimately managing, a research library and information service for approximately 200 research staff. Responsibilities will include the acquisition, organisation, classification and evaluation of books and periodicals combined with a computerised bibliographical and data base searching facility.

Applications are invited from men and women who have a 1st degree in either Chemistry or Biomedical Science and postgraduate qualification in information science combined with a minimum of 3 years experience managing a scientific research library.

If you are confident of being able to make a significant contribution to this prestigious research endeavour, then write enclosing full C.V. to Andrew Harley, Personnel Manager, Merck Sharp & Dohme Research Laboratories, Hertford Road, Hoddesdon, Herts EN11 9BU.

MSD MERCK SHARP DOHME (2893)A

Neuroscience Research Centre

Research Opportunities Medicinal Chemistry

Merck Sharp & Dohme Research Laboratories is a leading international pharmaceutical research organisation which is establishing a Neuroscience Research Centre on a 30 acre greenfield site near Harlow, Essex. The Centre will be one of the world's largest laboratories dedicated to neuroscience research initially focussing on the study of inhibitory chemical messengers in the brain and exploration of the functional role of neuropeptides in the control of emotion, appetite, memory and learning.

In preparation for the move to the Harlow site, the current eminent research team is to be expanded by the development of a Medicinal Chemistry group. In this respect the following positions have been created:

SENIOR RESEARCH CHEMIST Candidates for this career appointment should have a Ph.D in Chemistry with postdoctoral experience in organic synthesis.

POSTDOCTORAL FELLOW This appointment will be to carry out basic research in the peptide chemistry area. Experience of working in this field although advantageous, is not essential.

Our goal is to provide the facilities and compensations to enable the candidates appointed to devote their total endeavours to our research aims. If you feel that you meet our requirements write enclosing a full Curriculum Vitae stating current salary and listing three referees to: Andrew Harley, Personnel Manager, Merck Sharp & Dohme Research Laboratories, Hertford Road, Hoddesdon, Herts, EN11 9BU.

MSD MERCK SHARP DOHME (2894)A

Neuroscience Research Centre

Amgueddfa Genedlaethol Cymru — National Museum of Wales

Department of Botany

Research Assistant
CRYPTOGAMIC BOTANY

A Cryptogamic Botanist (non-bryological) is required to assist in the development and curation of collections in the Cryptogamic Section of the Department of Botany.

Applicants should have a broad taxonomic knowledge and a speciality in which he/she has the potential to make original contributions to taxonomy. The successful candidate will be appointed on the Research Assistant II (Curatorial Group F) salary scale (£6,133-£8,563) with the prospects of promotion to Research Assistant I (Curatorial Group E) salary scale (maximum salary £10,831), both salary scales under review.

Applicants must be unemployed.

Further particulars of the post may be obtained from the Secretary, National Museum of Wales, Cathays Park, Cardiff, CF1 3NP to whom applications should be submitted not later than 2 July 1984. (2351)A

THE INSTITUTE OF ZOOLOGY THE ZOOLOGICAL SOCIETY OF LONDON

LABORATORY TECHNICIAN

required to run a radioimmunoassay laboratory and to assist in studies on mammalian reproductive physiology. Candidates must have a training in biochemistry or endocrinology, at least 5 years' relevant experience and an H.N.C. or equivalent qualification. The appointment is for five years initially and salary, according to age, qualifications and experience, will be within the range £7794 — £8880 inclusive of London weighting allowance.

Please apply in writing by providing c.v. and names and addresses of two referees to the Establishment Officer, Zoological Society of London, Regent's Park, London NW1 4RY. (2338)A

MISCELLANEOUS

SPINCO ANALYTICAL CENTRIFUGE MODEL E

with Schlieren and UV optics (no scanner) to give away to *third world country*. Recipient must take over cost of transport to new location.

Inquiries to: Zürich 01 377 3403. (W1048)J

BEEBSTAT STATISTICS ON THE BBC MICROCOMPUTER

BEEBSTAT is a suite of eight programmes for standard statistical tests such as correlation and analysis of variance (several models). Data may be edited and transformed, kept on disc files and printed.

Details from Dominic Design Software, 19 Cressy House, Queens Ride, London SW13 0HZ. (2885)J

ASSISTANTSHIPS

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

Applications are invited for a POST-DOCTORAL RESEARCH ASSISTANTSHIP

in molecular biology for work on the development of plant gene vectors from virus replicons, involving DNA cloning and sequencing, transcriptional mapping, in vitro translation and plant cell transformation. The appointment will be for 2 years from 1 October 1984 with the possibility of renewal for a further period. Salary in the range £8376-£9761 including London Allowance.

Applications, with names of two referees, to Dr K W Buck and Dr R F A Coutts, Department of Pure and Applied Biology, Imperial College London SW7 2BB. (2121)P

Continued on page 2

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL LONDON

DEPARTMENT OF PHYSIOLOGY

LECTURESHIP

Applications are invited for a Lectureship in Physiology. Applicants should have an established record in research and would be expected to develop their own research interests.

The post will be held within the Joint Department of Physiology at University College London and this Medical School, where the post will be based.

Salary, according to age, qualifications and experience, within the range £8,376 — £15,312 inclusive of London Allowance.

Further information may be obtained from Professor J A Stephens, Sherrington School of Physiology, St Thomas's Hospital Medical School, London SE1 7EH tel 01-928 9292 ext 2241/7.

Applications in the form of a curriculum vitae, statement of research interests and the names and addresses of 3 referees should be sent to The Secretary, The Middlesex Hospital Medical School, London W1P 7PN by 19 July 1984. (2369)

UNIVERSITY OF YORK

DEPARTMENT OF BIOLOGY

RESEARCH ASSISTANT

Applications are invited for the post of research assistant in the Department of Biology, tenable for a period of three years from 1 September 1984, to work with Dr S M Bougourd on the effects of cancer chemotherapeutic drugs and tumour promoting agents on the chromosomes of human cell lines. The project is supported by the Yorkshire Cancer Research Campaign.

Applicants should have, or expect to obtain, a first or second class honours degree in a biological subject. Experience in cell culture techniques and cytogenetics is desirable but not essential. The successful candidate may have the opportunity to register for a higher degree.

Salary within the range £6,310 to £7,630 per annum, with USS.

Two copies of applications, with full curriculum vitae and naming two referees, should be sent to Registrar's Department (Appointments) University of York, Heslington, York YO1 5DD, by 11 July 1984. Further particulars are available. Please quote reference number 7120. (2321)A

UNIVERSITY OF OXFORD

Sir William Dunn School of Pathology

ADMINISTRATOR

Grade IIx.

Salary: £11,160-£15,085

Applications are invited from suitably qualified persons for the above post which it is hoped to fill from 1st September, 1984. The post carries superannuation in the USS. The Administrator, reporting directly to the Head of Department, is responsible for financial and budgetary control of the Department, for administration of the building, its services and research-support facilities and for the appointment and general supervision of technical, clerical and maintenance staff.

Further particulars may be obtained from Professor H Harris, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE to whom applications, giving details of education, qualifications and experience, together with the names and addresses of two referees should be sent as soon as possible. There is no application forms. (2353)A

PAPUA NEW GUINEA INSTITUTE OF MEDICAL RESEARCH

RESEARCH FELLOWS IN EPEDIMIOLOGY, BACTERIOLOGY, VIROLOGY, IMMUNOLOGY and BIOSTATISTICS

The Papua New Guinea Institute of Medical Research is a Statutory Body of the Papua New Guinea Government set up to conduct research into the disease problems affecting Papua New Guineans. Major research programs have been established for the principal diseases of pneumonia, malaria, diarrhoea and malnutrition, problems common to the countries of the tropical world.

The Institute was founded in 1968. It has its headquarters at Goroka in the Eastern Highlands of Papua New Guinea, with a major branch at Madang, on the north coast, and a smaller branch in the Southern Highlands Province. The Institute has a new laboratory and office complex in Goroka and laboratories, including an insectary in Madang. It has a total staff of over 100. Data processing is carried out with computers in Goroka, Madang and Mendi in the Southern Highlands, each of which has its own manager.

Each of the major research programs has an epidemiological base in rural village communities, with associated laboratory studies. Each program is an integrated investigation of mortality, morbidity, aetiology, pathogenesis, genetics and specific risk factors in defined populations, with the evaluation of an innovative intervention aimed at prevention or early treatment of the disease under study. Each investigation is collaborative with colleagues from overseas laboratories and is carried out under the direction of the Institute Director. Apart from its principal funding from the Government of Papua New Guinea the Institute receives support for its programs from the World Health Organization and other international agencies.

The pneumonia research program investigates the major cause of death in children in Papua New Guinea and its integrated approach has been used as a model for similar programs elsewhere in the tropical world. The interaction between viruses, bacteria (principally *Streptococcus pneumoniae* and *Haemophilus influenzae*), the immune response and other host factors in determining the severity of ubiquitous acute respiratory infections and the spread from upper to lower respiratory tract is being investigated. A pneumococcal vaccine trial is in progress.

The diarrhoea research program investigates the aetiology of diarrhoea and the interaction between pathogens, including parasites (the Institute has its own parasitologist), and host and environmental factors. An attempt is being made to introduce oral rehydration methodology at the community level. The diarrhoea research program is being carried out in close collaboration with the pneumonia research program in both field and laboratory aspects.

The malaria research program is defining the epidemiology of malaria in a holo/hyperendemic area and investigating the causes for small-area variation. Parasites are being cultured in vitro and antigenic diversity investigated. The immune response to malaria is being dissected. The ultimate thrust of the program is the development, in collaboration with colleagues overseas, of antimalarial vaccines derived from defined antigens and in establishing populations suitable for the field evaluation of such vaccines.

Applications are sought for the following positions:

1. Research Fellow in Epidemiology in the Diarrhoea Research Program in Goroka, now vacant;
2. Research Fellow in Epidemilogy in the Tari Research Unit, Southern Highlands Province, investigating pneumonia, diarrhoea and nutrition, vacant in January 1985;
3. Research Fellow in Epidemiology in the Malaria Research Program in Madang, vacant in April 1985;
4. Research Fellow in Bacteriology in the Diarrhoea Research Program in Goroka, now vacant;
5. Research Fellow in Bacteriology in the Pneumonia Research Program in Goroka, vacant in October 1984;
6. Research Fellow in Virology in the Diarrhoea Research Program in Goroka, now vacant;
7. Research Fellow in Immunology in the Pneumonia Research Program in Goroka, now vacant; and
8. Research Fellow in Biostatistics, stationed in Goroka but responsible for statistical aspects of all research programs, now vacant.

Research Fellows are sought with a higher qualification in their respective field at PhD or MSc level, with suitable experience. Those with technical qualifications and relevant experience may apply. Salaries are in the range of K16,820 to K21,520 depending on qualifications and experience. Salaries are subject to CPI indexation. An additional allowance of K2000 is payable to those with clinical qualifications valid for medical registration in Papua New Guinea. One kina (K1.00) = U.S. $1.20. In addition to salary a gratuity of 24% of salary earned is payable each year (increasing to 32% after 3 years' service). Taxation on salary is at standard levels but the gratuity is taxed at a flat rate of 2%. Contracts are normally for three years, but may be given for two years, and are renewable. Economy air fares are paid for the employee and family between the place of residence and Papua New Guinea on recruitment and repatriation. In addition, return leave fares for the whole family are payable every eighteen months to place of residence. Free accommodation is provided in Papua New Guinea. Education expenses are paid in Papua New Guinea. For children attending high school overseas, education allowances are paid and a fare from school to Papua New Guinea payable twice a year. Settling-in and settling-out allowances are paid at the beginning and end of the contract period.

Applicants should send a full curriculum vitae, including details of qualifications and experience, and the names and addresses of three referees to

Dr Michael Alpers
Director
Papua New Guinea Institute of Medical Research
P.O. Box 60, Goroka, EHP
Papua New Guinea

by 31 July 1984.

(W1054)A

APPOINTMENTS WANTED

advertise your qualities and qualifications through the most influential science weekly in the world at a special reduced cost of *25p a word.* (Personal Box Numbers £2). Advertisements must be pre-paid and sent to: Nature Classified (Appts Wtd), 4 Little Essex Street, London WC2R 3LF. (890)B

PHYSICIST (29) BSc 1st Class Honours, MSc, PhD, 4 years research experience. Background in X-rays, Neutron Diffraction, Mössbauer spectroscopy, IR, AES, Crystal Growth, Solid State Theory, Computer Programming. Seeks research position anywhere in the world. Available October 1984. Box 2344B c/o 4 Little Essex Street, London WC2R 3LF. (2344)B

STUDENTSHIPS

THE UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF BIOCHEMISTRY

SERC/CASE STUDENTSHIP

Applications are invited for a three year Ph.D. studentship starting October 1984 to work on the roles of lipid peroxidation and the covalent binding of drug metabolites in the development of liver toxicity.

The award is held cooperatively with Dr B Clark, Fisons Ltd., Pharmaceutical Division, Loughborough. Applicants must obtain a 1st or II(i) degree. Full curriculum vitae with names of two referees to Dr A A Horton, Department of Biochemistry, University of Birmingham, Birmingham B15 2TT. (2371)F

ROBERT GORDON'S INSTITUTE OF TECHNOLOGY

SCHOOL OF PHARMACY

RESEARCH STUDENTSHIPS IN PHARMACY AND PHARMACOLOGY

Applications are invited from graduates or students about to graduate with a 1st or 2nd Class Honours Degree in Pharmacy, Pharmacology, Biochemistry or Microbiology. Topics include

1. Mode of Action of DMSO

2. Characterisation of multiple-phase and oil-in-oil emulsions to evaluate their potential as vehicles for the controlled release of drugs

3. Mode of action of pharmaceutical preservatives on selected microbial species

4. The binding of anti-aggregatory compounds to , acid glycoprotein and to platelets

5. Involvement of products of arachidonic acid metabolism in platelet aggregation and bronchoconstriction.

Further details from Secretary, Robert Gordon's Institute of Technology, Schoolhill, Aberdeen AB9 1FR. (2895)F

OXFORD UNIVERSITY RESEARCH LABORATORY FOR ARCHAEOLOGY

Carbon-14 dating using a 2 MV tandem accelerator, thermoluminescence dating, archaeomagnetism: studentship(s) for DPhil research, suitable for physicists, chemists, earth scientists, anticipating a good honours degree.

Details: Dr M Aitken or Dr R Hedges, 6 Keble Road, Oxford OX1 3QJ, telephone 55211. (2347)F

ROTHAMSTED EXPERIMENTAL STATION Harpenden, Herts AL5 2JQ

PHYSIOLOGY & ENVIRONMENTAL PHYSICS DEPARTMENT

SERC CASE STUDENTSHIP: MATHEMATICAL MODELLING OF THE DRIFT OF AGRICULTURAL SPRAYS

Applications are invited for a SERC CASE Studentship tenable at Rothamsted Experimental Station and Imperial College of Science and Technology to study the drift of agricultural sprays downwind from crops. The project will involve field experiments and the use and adaptation of air pollution dispersion models.

Candidates should hold, or expect to obtain, a First or Upper Second Class Degree in Physics, Meteorology, Applied Mathematics or an Environmental Science.

Applications giving full cv and the names of two referees should be sent to: Dr H A McCartney, Physiology & Environmental Physics Department, Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ by 1st June 1984 quoting Ref: 557.

We are equal opportunities employers. (2367)F

FACULTY OF SCIENCE

SERC RESEARCH STUDENTSHIPS IN BIOLOGICAL SCIENCES

(2 Posts)

Required to work in one of the following areas of research: Fish biotechnology/immunology; Plant biotechnology; Physiological adaptations of marine crustaceans; Treatment and control of parasitic diseases of fish

Candidates should hold or expect to gain this year a good Honours degree in Biological Sciences.

Further details and application forms may be obtained from:-Department of Biological Sciences, Plymouth Polytechnic, Drake Circus, Plymouth PL4 8AA. Tel: 0752 264659. (2877)F

Plymouth Polytechnic

UNIVERSITY OF READING

DEPARTMENT OF MICROBIOLOGY

AFRC CASE STUDENTSHIP

Applications are invited from graduates with a First or Upper Second Class Honours degree in Microbiology or a related subject or from those who expect to be awarded such a degree this year for a three year AFRC CASE studentship commencing in October 1984. The project, in collaboration with Cadbury Schweppes, will be to develop a method for the detection of small numbers of yeasts which cause spoilage of soft drinks, using monoclonal antibodies. The project will be supervised by Dr A I Tiffin (Reading) and Dr C S Gutteridge (Cadbury-Schweppes).

Further particulars may be obtained from Dr A I Tiffin, Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ, to whom completed applications, a CV and the names of two academic referees should be submitted by 28 June, 1984. (2349)F

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF BIOCHEMISTRY

SGRC STUDENTSHIP IN STEROID BIOCHEMISTRY

Applications are invited for the above studentship, tenable for three years commencing October 1984. Candidates must hold, or expect to obtain, a I or II(i) Honours degree in an appropriate subject, and will be expected to register for a higher degree.

The project involves studying the relationship between sex steroids and the endometrium, particularly with respect to implantation, in cases of sub-fertility. The work will be carried out in the Department of Obstetrics and Gynaecology, but the student will also be a member of the Department of Biochemistry.

Applicants should write, giving names and addresses of two referees and enclosing a curriculum vitae to Dr P V Maynard, Department of Obstetrics and Gynaecology, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH. (2325)F

UNIVERSITY OF ABERDEEN

INSTITUTE OF ENVIRONMENTAL AND OFFSHORE MEDICINE

POSTGRADUATE STUDENTSHIP

Applications are invited from suitably qualified graduates in physiology, pharmacology or related biological sciences for a postgraduate studentship in the Institute. The field of study will be hyperbaric pulmonary oxygen toxicity and the objective will be the determination of safe oxygen exposure limits in diverse. It is anticipated that research will involve both human volunteers and animal models.

The studentship is available at normal Research Council rates from 1 October 1984. Applications including CV and the names of two referees should reach Dr T Shields, Institute of Offshore and Environmental Medicine, Aberdeen, AB9 2ZD, as soon as possible but by 6 July at the latest. (2360)F

UNIVERSITY OF READING SERC-CASE STUDENTSHIP IN MICROBIOLOGY

Applications are invited for an SERC-CASE Studentship, in co-operation with ICI, tenable for 3 years from October 1984 in the Department of Microbiology. Applicants must have, or expect to receive this summer, a 1 or 2i honours degree in microbiology or a closely related subject and have a particular interest in the physiology and biochemistry of microorganisms. The successful applicant will investigate the effect of CO_2 on the growth and metabolism of methylotrophic bacteria growing on methanol (eg the organism used for single-cell protein by ICI) and will register for a higher degree. About 3 months will be spent at the ICI Research Laboratories during the 3 years. The value of the Studentship is £2,430 pa tax free (under review), plus University fees, plus £600 pa and some travel expenses from ICI.

Applications with full curriculum vitae including a telephone number and two academic referees should be sent before 29 June 1984 to Dr L J Zatman, Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ. (2318)F

UNIVERSITY OF ABERDEEN

INSTITUTE OF ENVIRONMENTAL AND OFFSHORE MEDICINE

POSTGRADUATE STUDENTSHIP IN HUMAN PHYSIOLOGY

Applications are invited for a postgraduate studentship within the Institute. The project will investigate the role of substrate availability as a limiting factor in exercise performance, and will involve collection and analysis of blood and skeletal muscle samples from human volunteers. The effect of artificially induced variations in substrate availability on the metabolic response to exercise will be studied.

Applicants should have an interest in the physiology and biochemistry of exercise and should possess, or expect to obtain, a good honours degree in an appropriate discipline.

The Studentship is available at normal Research Council rates for a 3 year period from 1st October 1984. Applications including CV and the names of two referees, should reach Dr R Maughan, Institute of Offshore and Environmental Medicine, Foresterhill, AB9 2ZD, as soon as possible but by 6th July at the latest. (2361)F

STUDENTSHIPS *Continued*

THE UNIVERSITY OF OXFORD
Dyson Perrins Laboratory

SCIENCE AND ENGINEERING RESEARCH COUNCIL CASE STUDENTSHIP

Applications are invited for a CASE studentship leading to the degree of D Phil in organic chemistry to be held in the above department and sponsored by Shell Research Limited, Sittingbourne, Kent. The successful applicant will work with Dr L M Harwood on the isolation and synthesis of certain allelophathic agents from plant sources. Applicants should hold or expect to gain this year a good honours degree in chemistry and have a particular interest in organic synthesis.

It is intended that the studentship will be taken up by 1st October, 1984 and applications (including a curriculum vitae and quoting two referees) should be sent to Dr L M Harwood, the Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY. (2312)F

UNIVERSITY OF CAMBRIDGE

Applications are invited for

RESEARCH STUDENTSHIPS

for the PhD, one each in insect chemistry; nutrients of potatoes; establishment of wheat and two in soil processes.

Further details can be obtained from Professor Sir James Beament, Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX. (2315)F

THE HATFIELD POLYTECHNIC

SCHOOL OF NATURAL SCIENCES

RESEARCH STUDENTSHIPS IN BIOLOGY

A SERC Studentship is available for one of the following projects:-

(i) cell-free transcription of exogenous plant DNA.

(ii) effects of morphological differentiation of Streptomyces spp. on antibiotic production.

(iii) identification of different races of Plasmodiophora brassicae.

A Research Assistantship is also available to work on the biotechnology of abattoir waste. This project will investigate microbiological and biochemical characteristics of abattoir waste and its potential for conversion into high value byproducts. Applicants for the post must possess a driving licence.

Applicants for both posts should possess a good honours degree in an appropriate subject and should submit a full Curriculum vitae and the names of two academic referees to Dr C Wilson, Biological Sciences, The Hatfield Polytechnic, P O Box 109, Hatfield, Herts. AL10 9AB (Hatfield 68100, Ext. 481). Full details of the projects are available on request.

Closing date: 9 July 1984 (2357)F

UNIVERSITY OF READING

NATIONAL COLLEGE OF FOOD TECHNOLOGY

AFRC STUDENTSHIP

Applications are invited from graduates who hold or expect to obtain first or upper second class honours degrees to work on either:

a) Glycosyl Transferases or

b) Physicochemical Aspects of Food Processing.

Candidates for project (a) should be graduates in biochemistry, microbiology, chemistry, food science or related subjects. Candidates for project (b) could also have degrees in engineering or applied physics.

Applications including a CV and names of two referees should be sent to Dr G G Birch, National College of Food Technology, Food Studies Building, University of Reading, Whiteknights, Reading, RG6 2AP. (2341)F

UNIVERSITY OF READING

DEPARTMENT OF PURE & APPLIED ZOOLOGY

Applications are invited for suitably qualified graduates for an

SERC STUDENTSHIP

to undertake research for a higher degree in the areas of comparative physiology, behaviour, parasitology or applied entomology.

Applicants should write to Professor K Simkiss, Department of Pure & Applied Zoology, Whiteknights, University of Reading, RG6 2AJ, stating their interests and enclosing a curriculum vitae with the names of 2 referees. (2316)F

WELSH NATIONAL SCHOOL OF MEDICINE
(University of Wales)

DEPARTMENT OF MEDICAL BIOCHEMISTRY

PHD STUDENTSHIP

Applications are invited for a Research Studentship (supported by the Welsh Scheme for the Development of Health and Social Research) tenable for three years commencing October 1984. The successful applicant will work under the supervision of Dr M A McPherson on biochemical control of exocrine secretion; with the aim of investigating abnormal regulatory factors(s) which may result from expression of the genetic defect in cystic fibrosis.

Candidates should have or expect to obtain a good Honours degree in Biochemistry or a closely-related subject.

Applicants should apply in writing, including a curriculum vitae with the names and addresses of two academic referees (quoting Ref No C38/4/50) to the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN (Tel No 0222/755944 Ext 2296) from whom further details are available. Closing date for application: 31st July, 1984. (2350)F

UNIVERSITY OF READING

DEPARTMENT OF AGRICULTURE AND HORTICULTURE

MAFF RESEARCH STUDENTSHIP

A MAFF research studentship is available from 1 October 1984 for a project on "The compensatory threshholds/tolerance limits of cereals to invasion by nematodes". The work will involve the use of growth regulators to alter the morphology of cereal roots and hence the potential invasion sites.

Applicants should send CV and the names of two referees to Dr N G M Hague, Department of Agriculture and Horticulture, Earley Gate, University of Reading, Whiteknights, Reading, RG6 2AT. (2342)F

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF ZOOLOGY

Research Studentships are available in at least two of the following fields: cell motility, the cytoskeleton of protozoans, cell surface receptors, insect parasitology, ecological genetics, avian ecology and the ultrastructure of lower vertebrates.

Further particulars are available from Dr D S O'Dell, Postgraduate Tutor, Department of Zoology, University College London, Gower Street, London WC1E 6BT (01-387 7050 Ext 424). Applications should include the names of two academic referees. (2887)F

UNIVERSITY OF READING

RESEARCH STUDENTSHIP IN MICROBIAL PHYSIOLOGY & BIOCHEMISTRY

Applications are invited for a Research Studentship tenable for 3 years from October 1984 in the Department of Microbiology. Applicants must have, or expect to receive this summer, a 1 or 2i honours degree in microbiology or a closely related subject and have a particular interest in the physiology and biochemistry of microorganisms. The successful applicant will register for a higher degree and will investigate the mechanism of nitrogen assimilation by microorganisms from natural habitats followed by physiological, biochemical and enzymological studies. Up to 64 hours per term demonstrating duties will be required for which extra payment will be made. The value of the Studentship is £2,430 pa tax-free (under review), plus approx £300 per term for demonstrating duties, plus University fees.

Applications with full curriculum vitae including a telephone number and two academic referees should be sent before 29 June 1984 to Dr L J Zatman, Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ. (2317)F

UNIVERSITY OF ABERDEEN

DEPARTMENT OF OPHTHALMOLOGY

POSTGRADUATE STUDENTSHIP

Applications are invited from suitably qualified candidates for a 3-year postgraduate studentship to work on immunological aspects of ocular inflammatory disease, using an established model of experimental allergic uveo-retinitis (EAU). The work involves the use of purified retinal S antigen and is aimed at determining the relationship between altered immune responses to the antigen and severity of the disease.

The successful applicant will work in the new University Department of Ophthalmology and will be expected to participate in its development. Applicants should have a 1st or upper 2nd class Honours Degree in the biological sciences. Experience in biochemistry and/or immunology would be an advantage.

The Studentship will be at normal Research Council rates and is available immediately although it is expected to commence around October, 1984. Applications (together with with names of two referees) should be sent to Professor A Forrester, Department of Ophthalmology, University of Aberdeen, as soon as possible but by 6th July at the latest. (2362)F

UNIVERSITY OF LONDON
ST. GEORGE'S HOSPITAL MEDICAL SCHOOL

DEPARTMENT OF PHARMACOLOGY AND CLINICAL PHARMACOLOGY

SERC CASE AWARD STUDENTSHIP
In association with the Lilly Research Centre

Applications are invited from suitably qualified candidates (First or Upper Class Honours) for the above award tenable from October 1984.

The project, supervised by Professor J.S. Kelly and Dr S.R. Forda, involves the maintenance of 5HT containing neurones of the raphe nucleus in dissociated cell culture to provide a model in which the intrinsic activity of 5HT neurones can be examined. Electrophysiological, autoradiographic and immunocytochemical techniques will be used to identify the site and mechanism by which centrally active drugs of clinical importance, particularly antidepressants and analgesics, affect 5HT receptor function.

Applications, including brief curriculum vitae and the names of two referees should be sent to Professor J.S. Kelly, Department of Pharmacology and Clinical Pharmacology, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE.

Further details can be obtained by writing to Professor Kelly or by telephoning (01-627 1255 Ext. 4722). (2889)F

ASSOCIATESHIPS

UNIVERSITY OF BIRMINGHAM

DEPARTMENTS OF BIOCHEMISTRY AND ZOOLOGY AND COMPARATIVE PHYSIOLOGY

RESEARCH ASSOCIATESHIP

Applications are invited for the above post to work on the isolation and characterisation of naturally occurring factors which inhibit growth and oviposition in freshwater snail hosts of schistosome parasites.

It is anticipated that the successful candidate will hold at least an upper second class honours degree in Biochemistry or a related subject, and it would be possible to register for a higher degree.

The post is initially for one year but support for a further two years is envisaged, subject to satisfactory progress.

Salary on the Research Associate 1B scale £6,310 — £8,530 plus superannuation.

For further particulars phone 021-472 1301 ext 2559, quoting reference J3.

No formal application form. Three copies of application, including full Curriculum Vitae and naming three referees to Assistant Registrar, (Science and Engineering), PO Box 363, Birmingham B15 2TT by June 1984. (2320)O

ACCOMMODATION

SOUTH SUBURB Paris, furnished apartment for academic visitor — Aug 84 — Sept 85. Three bed rooms. Equipped kitchen — convenient for Paris, Orsay, Saclay. Meudon, Créteil, Villejuif. Dr Pierre Gibart, Le Hameau de Pré du Lac, F-06740 Chateau Neuf de Grasse (W1053)S

FELLOWSHIPS

UNIVERSITY OF EAST ANGLIA
Norwich

Applications are invited for a University Postdoctoral Fellowship in

ACID RAIN RESEARCH

The person appointed will work on *either*: a) Soil Microbiology — Acid Rain Interactions *or* b) Factors Controlling the pH of Rain in Remote Areas. Project a) requires someone with experience in soil microbiology, and project b) needs a person trained in chemistry and/or environmental chemistry. The appointment is for two years starting 1 October 1984, or as soon as possible thereafter. Salary on the scale £7,190 — £8,530 per annum plus USS (under review).

Further information is available from Dr T.D. Davies (project a)) or Dr P.S. Liss (project b)), School of Environmental Sciences, University of East Anglia, Norwich NR4 7TJ. Applications, including the names of two referees, should be sent as soon as possible to the Dean of the School of Environmental Sciences, University of East Anglia, Norwich NR4 7TJ. (2873)E

NIHE

NATIONAL INSTITUTE FOR HIGHER EDUCATION DUBLIN

POSTDOCTORAL FELLOWSHIP: GROWTH FACTORS IN CELL CULTURE

A fellowship exists, supported by the Irish Cancer Society, to work on genetic control of growth factor production by human tumour cells in culture. Experience in cell culture, recombinant DNA techniques or peptide separations would be an advantage. Applications to: Dr. M. Clynes, School of Biological Sciences, N.I.H.E., Glasnevin, Dublin 9, Ireland. (2876)E

UNIVERSITY OF EDINBURGH

DEPARTMENT OF ZOOLOGY

POST-DOCTORAL FELLOWSHIP IN BIOCHEMISTRY

Applications are invited for a SERC Fellowship to work on the biochemistry of the yeast cell cycle. Research range IA starting 1 October 1984.

Further particulars are available from Professor J M Mitchison, Department of Zoology, University of Edinburgh, Kings Buildings, West Mains Road, Edinburgh EH9 3JT with whom applications (2 copies) including CV and the names of two referees should be lodged not later than 9 July 1984.

Please quote reference No 5178. (2311)E

THE HATFIELD POLYTECHNIC

SCHOOL OF NATURAL SCIENCES

POST-DOCTORAL FELLOWSHIP IN BIOTECHNOLOGY

Applications are invited for a Post-Doctoral Fellowship to work on either:-

(i) the stability of plasmid vectors in yeast; or,

(ii) the molecular biology of algal viruses.

Both projects require expertise in recombinant DNA techniques. Experience in either yeast research or continuous culture would be an asset for the plasmid vector project and experience in animal or plant virus for the algal viruses project.

Full details of both projects may be obtained from Dr E GINGOLD, Biological Sciences, The Hatfield Polytechnic, P O Box 109, Hatfield, Herts. AL10 9AB (Hatfield 68100, Ext.489).

The appointment is for three years from October 1984. The salary is in the range of £7,215 - £9,801 + £258 Local Allowance (under review).

Applicants, who should possess a PhD or have submitted a thesis, should submit a full Curriculum vitae and the names of two academic referees to Dr Gingold.

Closing date for applications: 16 July 1984 (2356)E

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF MICROBIOLOGY

RESEARCH FELLOWSHIP

Applications invited for this MRC funded post. The Project is designed to purify and characterise enterotoxin(s) of Salmonella typhimurium as a prelude to detecting their production, location and release in vivo by immunoelectron microscopy. Work will be carried out in Departments of Microbiology and Institute of Child Health in conjunction with Department of Physiology of Birmingham University.

The post is tenable for up to three years from the soonest possible date.

Salary on the Research Fellow 1A scale £7,190-£11,615 plus superannuation.

For further particulars phone 021-472 1301 ext 2559, quoting reference M5.

No formal application form. Three copies of application, including full Curriculum Vitae and naming three referees to Assistant Registrar, (Science and Engineering), PO Box 363, Birmingham B15 2TT by 29 June, 1984. (2880)E

THE UNIVERSITY OF SYDNEY

ROTHMANS FELLOWSHIPS

Applications are invited for Rothmans Fellowships, which are awarded under the Rothmans University Endowment Fund set up by Rothmans of Pall Mall (Australia) Limited to enable Fellows to undertake postgraduate work within an Australian University.

Rothmans Fellowships are of an annual value of up to $A17,000. A Fellow may be paid travelling expenses incurred in taking up the Fellowship and returning home.

In addition, an amount of $A2,000 pa towards fees and expenses including the purchase and maintenance of equipment may be paid to the University where the Fellow is working.

A Fellow shall take up a Fellowship before attaining the age of twenty-eight. The Fellowships are open to graduates of any University who have had at least three years postgraduate experience in research. The Fellowships are not open to permanent members of academic staff or applicants proceeding on sabbatical, study or other leave (including leave without pay). The Fellowships must be held at an Australian University.

Application forms and further details may be obtained from The Secretary, Rothmans University Endowment Fund, c/o The University of Sydney, NSW 2006, Australia, or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications close on 29 July 1984. (W1060)E

UNIVERSITY OF BIRMINGHAM

DEPARTMENTS OF MICROBIOLOGY AND GENETICS

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for the above post which is funded by an MRC grant to Dr C W Penn (Microbiology) and Dr C M Thomas (Genetics). The work will involve cloning and expression of antigen genes of the syphilis agent Treponema pallidum in Escherichia coli. Candidates should have a background in microbiology and/or biochemistry and should preferably have some experience of recombinant DNA techniques, although this is not essential.

Post tenable for up to three years from 1/4/84 or as soon as possible thereafter.

Salary on the Research Fellow 1A scale £7,190-£11,615 plus superannuation.

For further particulars phone 021-472 1301 ext 2559, quoting reference M6.

No formal application form. Three copies of application, including full Curriculum Vitae and naming three referees to Assistant Registrar, (Science and Engineering), PO Box 363, Birmingham B15 2TT by 29 June 1984. (2879)E

UNIVERSITY OF WAIKATO

POST-DOCTORAL FELLOWSHIPS

(A 84/15)

The University of Waikato invites applications for post-doctoral fellowships to be taken up during 1985 for one or for three years. The fellowships will be funded and awarded by the University Grants Committee and tenable at the University of Waikato.

Applications from candidates who wish to hold a fellowship in any department of the University will be considered: the University Grants Committee, however, in making the awards, will give priority to current growth research areas such as biotechnology, genetic engineering, electronics, engineering, chemistry, etc.

Applicants should have been awarded, or qualified for the award of, the degree of Doctor of Philosophy.

The emolument payable to the Fellow will be determined at a rate not exceeding step one of the scale for Lecturers (ie NZ$21,660 p.a.).

Before lodging an application, prospective applicants should obtain details of the method of application and conditions of appointment from the Registrar, University of Waikato, Private Bag, Hamilton, New Zealand, or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF. Applications should be forwarded to the Registrar by 25 June 1984. (W1055)E

FELLOWSHIPS *continued*

Southampton THE UNIVERSITY

INSTITUTE OF CRYOGENICS RESEARCH FELLOWSHIP

Applications are invited for a 2 year research fellowship supported by SERC to study convection in cryogenic fluids using Laser Doppler Anemometry and microthermometry. Experience with data processing and lasers preferred.

Salary on range: £7,190-£8,080 per annum (under review).

Applications giving Curriculum Vitae (in duplicate) and names of two referees should be sent to D.A.S. Copland, Southampton University, Highfield, Southampton, SO9 5NH as soon as possible quoting reference 2056. (2340)E

Special Training Fellowships in Recombinant DNA Technology

Applications are invited for fellowships for any period from 6 months to three years commencing on or after 1 May 1985 from scientists requiring training in recombinant DNA techniques. Applicants may be young postdoctoral scientists (or those just completing PhD's) or more mature scientists with established reputations in other areas wishing to enter this field. Permanent academic staff with unpaid leave of absence may apply.

These fellowships are tenable in any UK institution offering training appropriate to the applicant's needs. UK residents currently working abroad may apply.

MRC Medical Research Council

Application forms, and further details may be obtained from Training Awards Group, Medical Research Council, 20 Park Crescent, London W1N 4AL. (Tel: 01-636 5422 Ext 448)

Closing date: 15 October 1984

(2896)E

THE RANK PRIZE FUNDS

Rank/Widdowson Post-Doctoral Fellowship in Child Nutrition

The Trustees of The Rank Prize Funds are to honour Dr. Elsie Widdowson, F.R.S. by establishing for three years a Post-Doctoral Fellowship at the M.R.C. Dunn Nutrition Unit to work on the metabolic and physiological aspects of infant nutrition.

Applicants should have a recently awarded Ph.D. in an appropriate biological science or expect to be awarded a Doctorate in the near future.

Details will be provided to short listed candidates.

Applicants should write to Dr. R. G. Whitehead, M.R.C. Dunn Nutrition Unit, Downhams Lane, Milton Road, Cambridge, CB4 1XJ giving details of their previous research experience and indicating the type of work which they would like to pursue in the field of infant nutrition.

The terms and conditions of service for this Fellowship will be identical with those of an M.R.C. Post-Doctoral Fellowship.

Applications should reach the Dunn Nutrition Unit by the 15th July, 1984. (2343)E

E M B O
European Molecular Biology Organization
LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY
AUTUMN 1984 AWARDS
Next deadline: August 15, 1984

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligibile a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are awarded initially for one year, but depending on a review of progress, they be renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is August 15, 1984. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on October 29, 1984.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F.R. Germany.

(W1016)E

ICI

NEW SCIENCE GROUP
POSTDOCTORAL RESEARCH FELLOWSHIP AT MIT

Applications are invited from suitably qualified candidates for an ICI New Science Group Postdoctoral Fellowship based within the Department of Materials Science at the Massachusetts Institute of Technology in Boston, USA. This prestigious appointment will be for two years with a starting date in September 1984. The research fellow will be involved in studies of sintering phenomena in ceramic precursor systems and will work in close association with Professor J.D. Birchall FRS at ICI's New Science Group in his capacity as Visiting Professor at MIT.

The fellowship offers a unique opportunity for carrying out original research with leading edge University and Industrial research laboratories. Applications are invited from candidates with a PhD in Physical or Inorganic Chemistry, Materials Science, Solid State Physics or Ceramics who feel that they could contribute to the aims of this fundamental research programme.

The value of the Fellowship is $23,000 per annum with travelling expenses to and from the UK at the start and finish of the fellowship being provided by ICI. It is anticipated that the Fellowship may well lead to employment with ICI New Science Group on completion.

Applicants should send a curriculum vitae and the names of three referees to Miss E. Lauermann, Personnel Officer, Imperial Chemical Industries PLC, New Science Group, P.O. Box 11, The Heath, Runcorn, Cheshire, marking the envelope MIT Fellowship. The closing date for applications is 28th June 1984.

NSG

(2314)E

GRANTS and SCHOLARSHIPS

Rainbow Research Scholarship

Bass Public Limited Company invite applications for the Rainbow Research Scholarship, which was founded in memory of the contribution to brewing science by the late Dr. C. Rainbow and which falls vacant in January 1985 for the second occasion. Candidates should hold a first or second class honours degree, preferably in genetics or a related subject.

The project entitled "Developing Systems for Genetic Manipulation of Brewing Yeast", is aimed at the generation of new vector systems for yeast and rendering *Saccharomyces* spp. more amenable to genetic manipulation. The successful candidate will be required to seek registration with the Council for National Academic Awards for the degree of Doctor of Philosophy and will spend a period of 3 years in the research laboratories at Burton-on-Trent, where working conditions and facilities are first-class. This constitutes a unique opportunity to acquire research training in a progressive industrial research environment.

We will meet all the costs of the project and associated fees. The candidate will have access to the comprehensive sporting and social facilities provided by the Company. Written application, curriculum vitae and the names of two referees should be addressed to:-

Director of Research,
Bass Public Limited Company,
137 High Street, Burton-on-Trent, DE14 1JZ.

(2319)H

Bass
Public Limited Company

AWARDS

British Diabetic Association/ St Bartholomew's Hospital

ANDREW CUDWORTH MEMORIAL PRIZE

To commemorate the work of the late Professor Andrew Cudworth, the British Diabetic Association, jointly with the Department of Medicine, St Bartholomew's Hospital, offers a prize of £250 for the best essay submitted on any aspect of diabetes mellitus. Essays should not exceed 10,000 words and the winner may be invited to present the work to one of the meetings of the Medical and Scientific Section for publication.

Applicants should be under age 40 and of British nationality. Four copies of the essay should be submitted for consideration to the Secretary General, British Diabetic Association, 10 Queen Anne Street, London, W1M OBD by 30th September 1984. **(2892)N**

ASSISTANTSHIPS

UNIVERSITY OF SOUTHAMPTON
FACULTY OF MATHEMATICAL STUDIES
RESEARCH ASSISTANTSHIP IN SEMI CONDUCTOR MODELLING

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Further particulars from, and applications (naming two referees) to: The University Secretary, University of Stirling, Stirling FK9 4LA. Tel: 73171, ext 2314. Closing date: 16 July 1984. (2346)P

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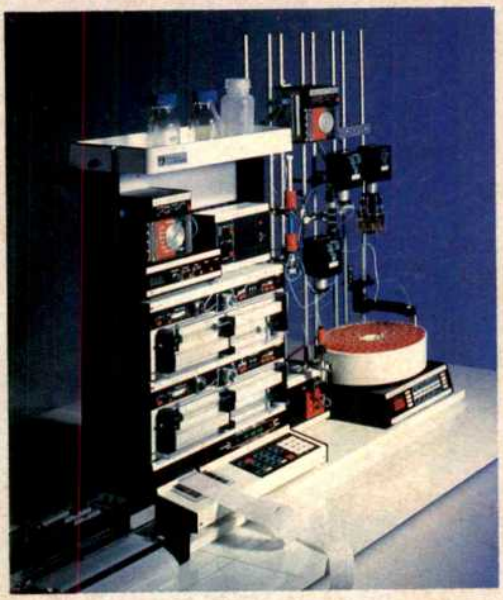
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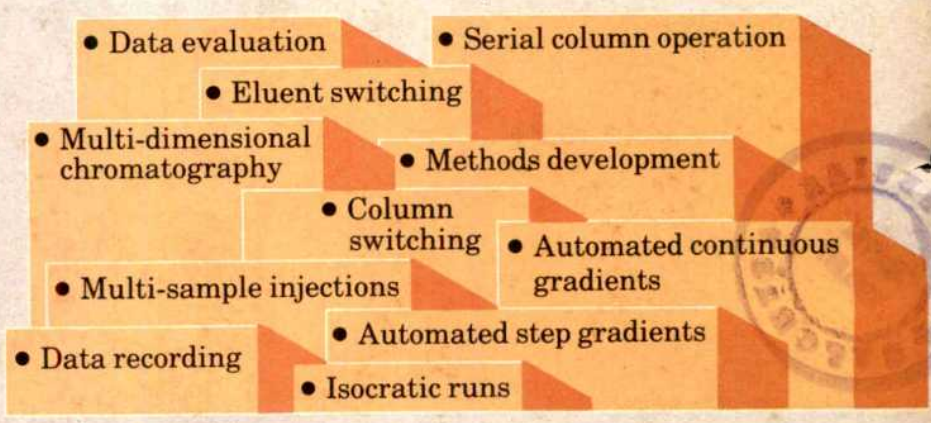
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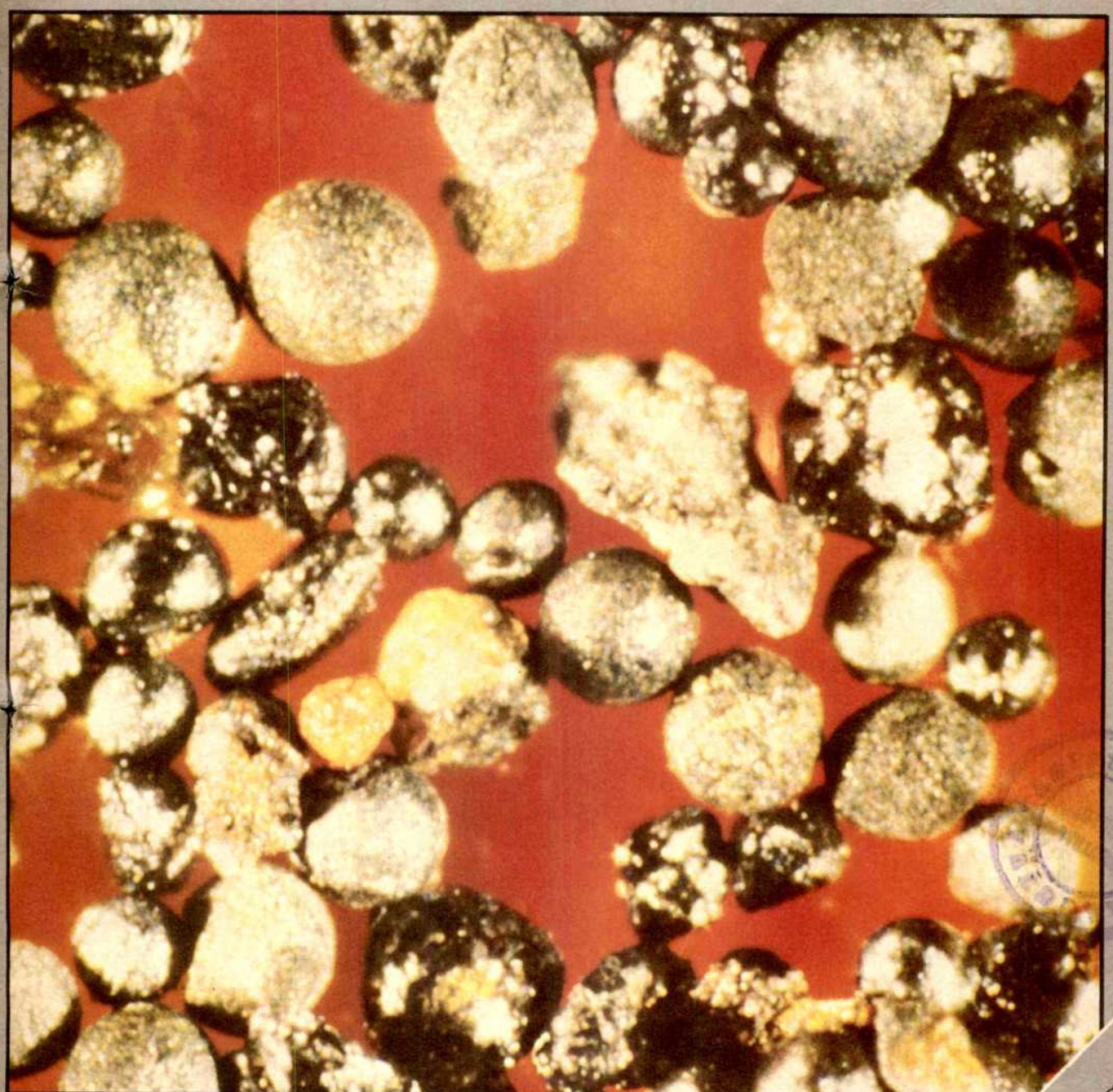
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CANCER

Conference Program
September 10-12, 1984
Boston Park Plaza Hotel

Daily Themes:

DAY 1 - ONCOGENES,
DAY 2 - THE CANCER CELL,
DAY 3 - PROSPECTS/PROBLEMS.

DAY 1

MONDAY, 10 September
Exhibition Viewing: 8:30am— 6:30pm
Recruitment Center: 9:30am— 6:00pm

ONCOGENES AND GROWTH FACTORS — WHAT THEY ARE AND WHAT THEY DO

9:00— 9:15	Chairman
9:15—10:00	Oncogenes *R. Weinberg (MIT)*
10:00—10:45	Growth factors *M. Sporn (NIH)*
11:15—12:00	HTLV *R. Gallo (NIH)*
12:00—12:30	Clinician's View *S. Broder (NIH)*
2:00— 4:00	Roundtables with morning speakers
4:30— 5:30	The Cancer Cell *J. Cairns (Harvard)*

DAY 2

TUESDAY, 11 September
Exhibition Viewing: 8:30am— 6:30pm
Recruitment Center: 8:30am— 6:00pm

THE CANCER CELL — HEREDITARY CANCER, TUMOR PROMOTERS AND CARCINOGENESIS

9:00— 9:15	Chairman
9:15—10:00	Gene Regulation *P. Leder (Harvard)*
10:00—10:45	Hereditary cancer *R. White (Utah)*
11:15—12:00	Carcinogens and promoters *I.B. Weinstein (Columbia)*
12:00—12:30	Clinician's View *F. Gilbert (Mt. Sinai)*
2:00— 4:00	Roundtables with morning speakers
4:30— 5:30	NCI program

DAY 3

WEDNESDAY, 12 September
Exhibition Viewing: 8:30am— 3:00pm
Recruitment Center: 8:30am— 3:00pm

PROSPECTS AND PROBLEMS— DRUG RESISTANCE, MONOCLONAL ANTIBODIES AND NEW DRUGS

9:00— 9:15	Chairman
9:15—10:00	Diagnostics and therapeutics *G. Todaro (Oncogen)*
10:00—10:45	Immunotoxins *J. Uhr (Texas)*
11:15—12:00	Drug resistance *A. Varshavsky (MIT)*
12:00—12:30	Clinician's View *J. Gutterman (Houston)*
2:00— 4:00	Roundtables with morning speakers
4:00— 4:45	Prospects
4:45	Closing remarks

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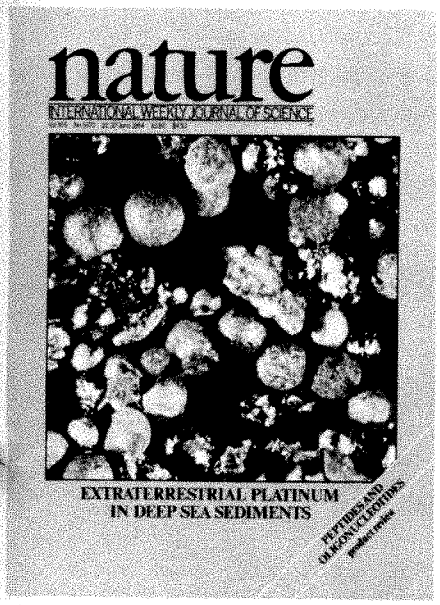
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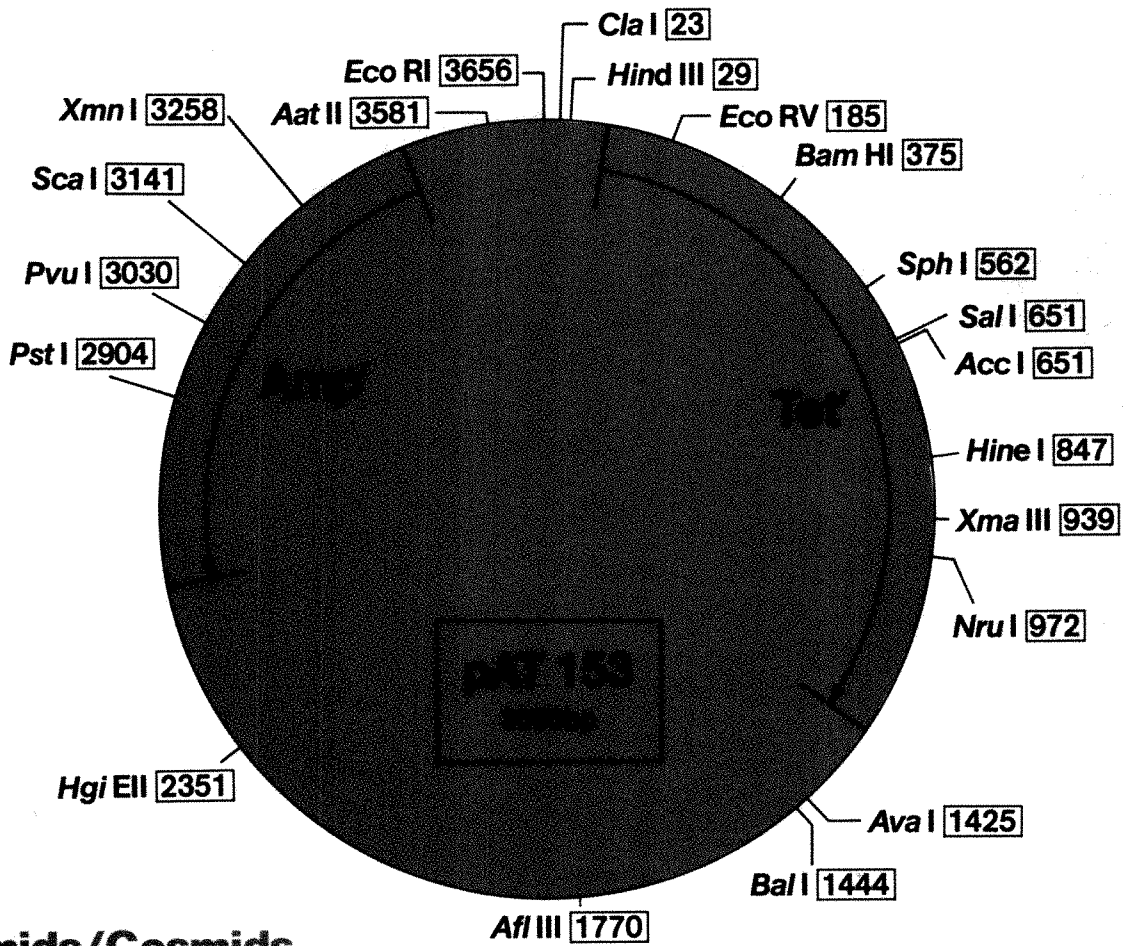
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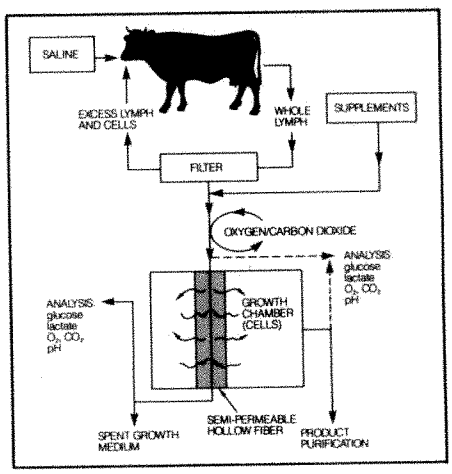
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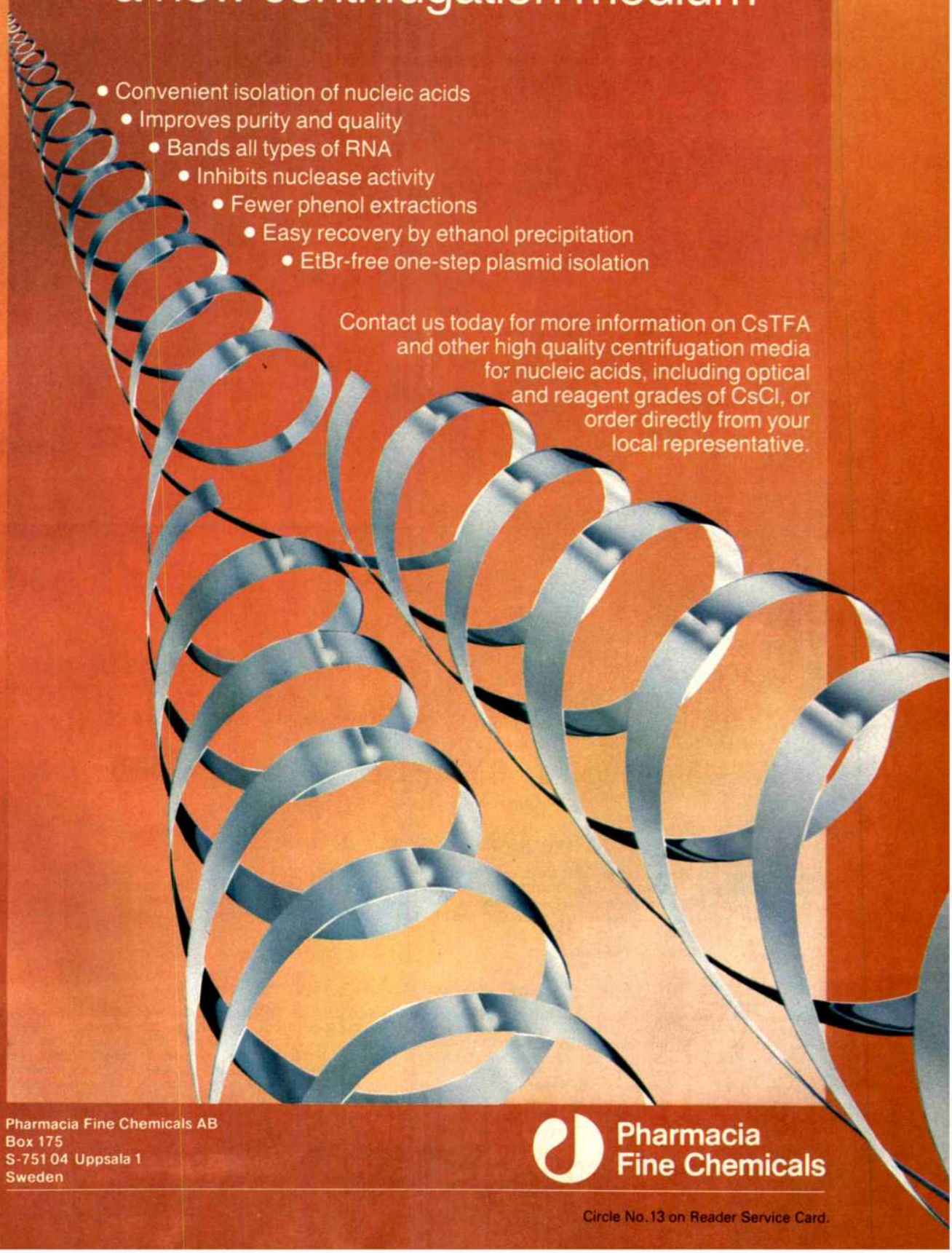
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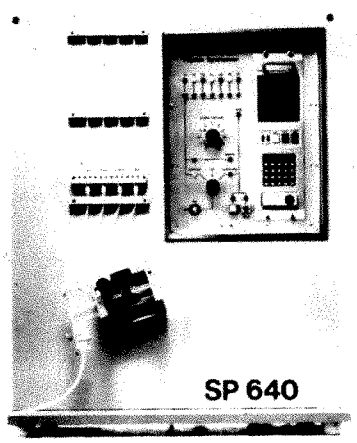
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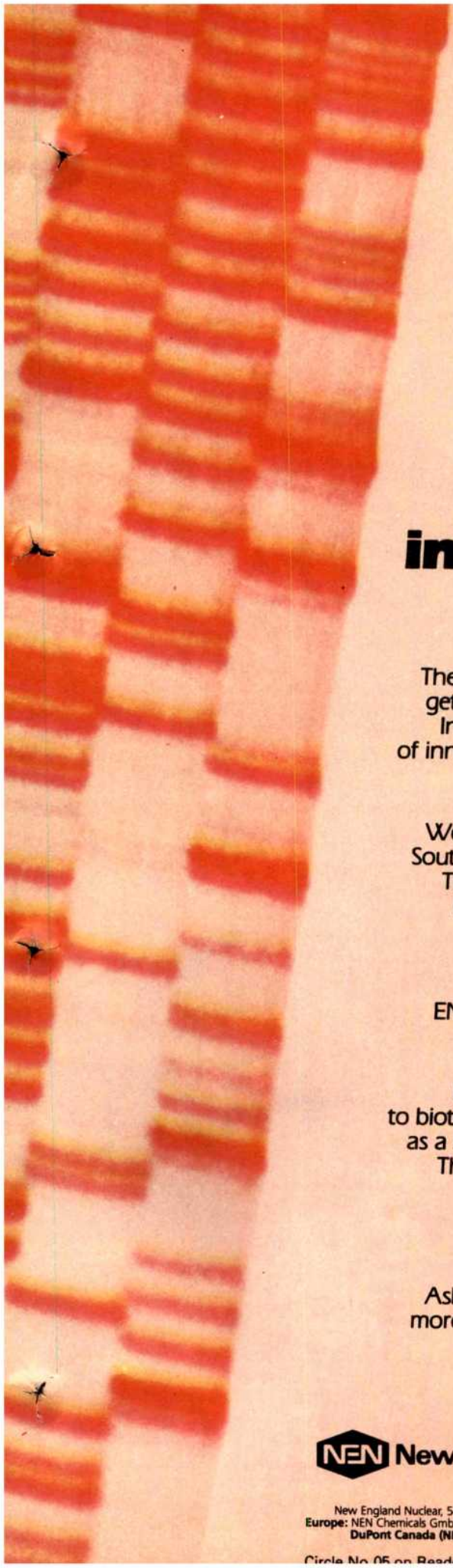
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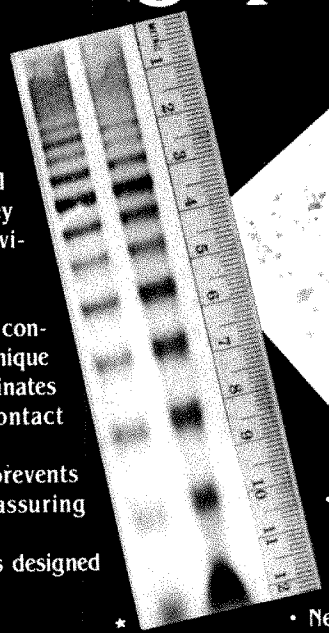


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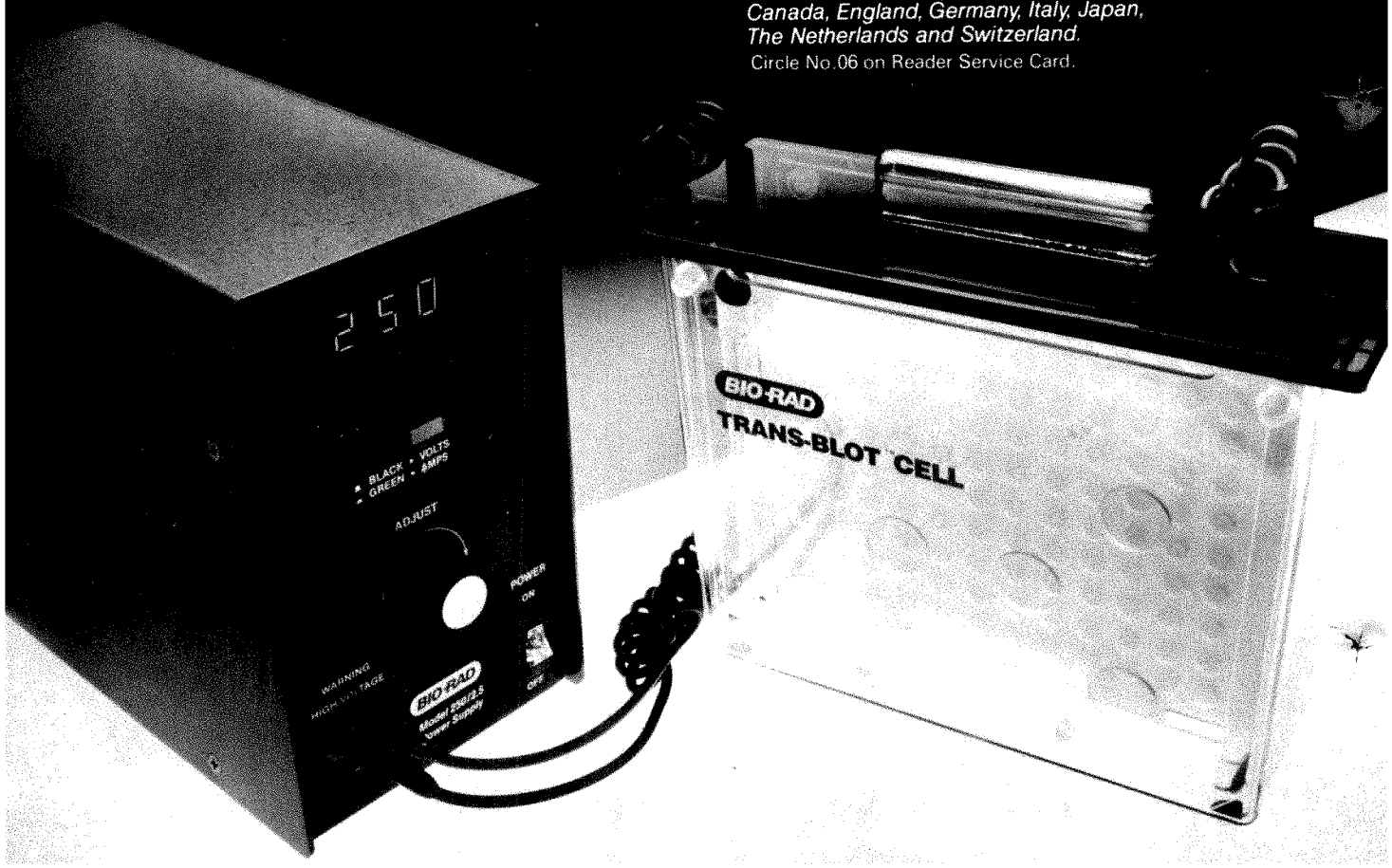
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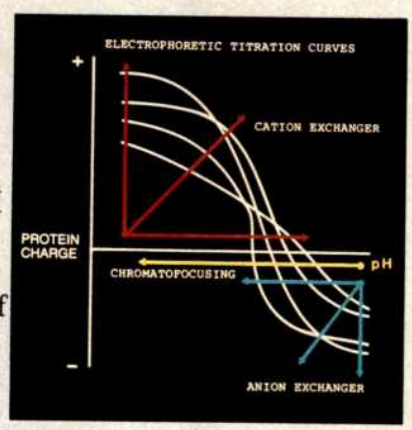


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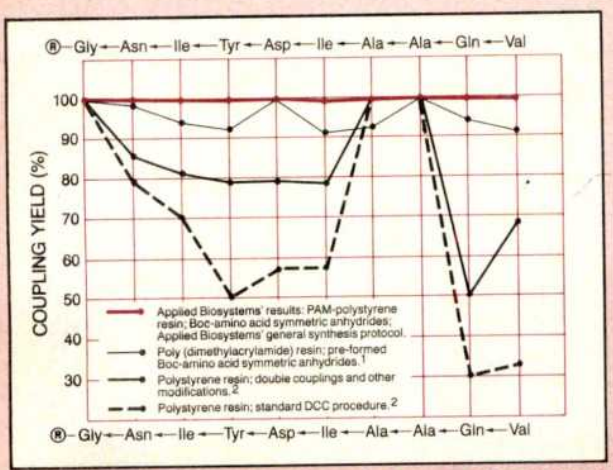
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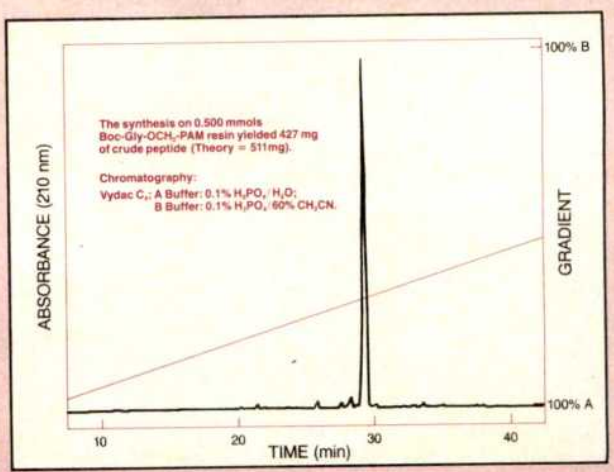
Cycle times with the general synthesis protocol are approximately one hour. A single loading of protected amino acids, reagents, and solvents will give up to 50 synthesis cycles. To insure high coupling yields, Applied Biosystems manufactures and supplies all synthesis reagents.

The data below summarize the results of the synthesis of the decapeptide Acyl Carrier Protein (65-74). These results illustrate the combined capabilities of the novel automated synthesis procedure and the high quality peptide synthesis reagents and loaded resins.

The new Model 430A Peptide Synthesizer was introduced at FASEB, Analytica and at the ASBC Meeting. For more information, circle No. 08



Amino acid incorporation during assembly of Acyl Carrier Protein residues 65-74.



HPLC chromatogram of crude, HF cleaved Acyl Carrier Protein (65-74).

ANALYTICAL METHOD	STEP YIELD (%)									
Quantitative Ninhydrin Monitoring[3].	—	99.9	99.6	99.5	99.4	99.1	99.2	99.2	99.1	98.9
Preview Quantitation by Solid Phase Sequencing of Protected, Resin Bound Peptide[4].	—	—	99.4	—	99.3	99.1	99.2	—	98.9	98.7
	RELATIVE AMINO ACID EQUIVALENTS									
Amino Acid Analysis of HF Cleaved, Deprotected Peptide	1.00	0.97	0.90	0.94	0.97	0.90	0.96	0.96	0.94	0.98
Amino Acid Residue	Gly	Asn	Ile	Tyr	Asp	Ile	Ala	Ala	Gln	Val

Step yield quantitation and amino acid analysis results for Acyl Carrier Protein (65-74) chain assembly using Applied Biosystems' general synthesis protocol. Only single couplings were used throughout the synthesis (except for Gln).

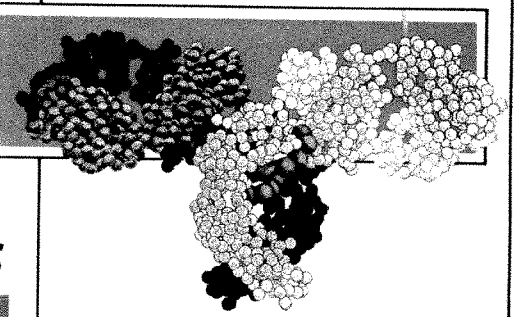
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4. Stephen B.H. Kent, Mark Rieman, Mary LeDoux and R.B. Merrifield, *Proc. Int'l. Conference: Methods of Protein Sequence Analysis*, 1982

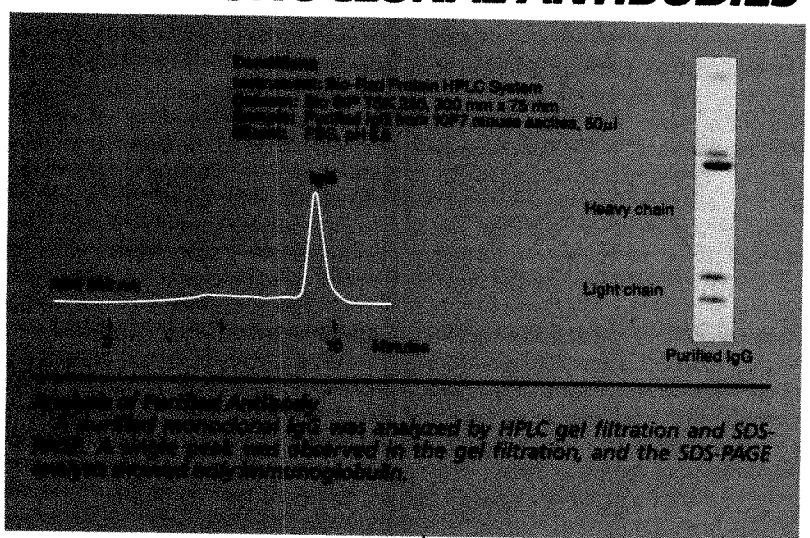
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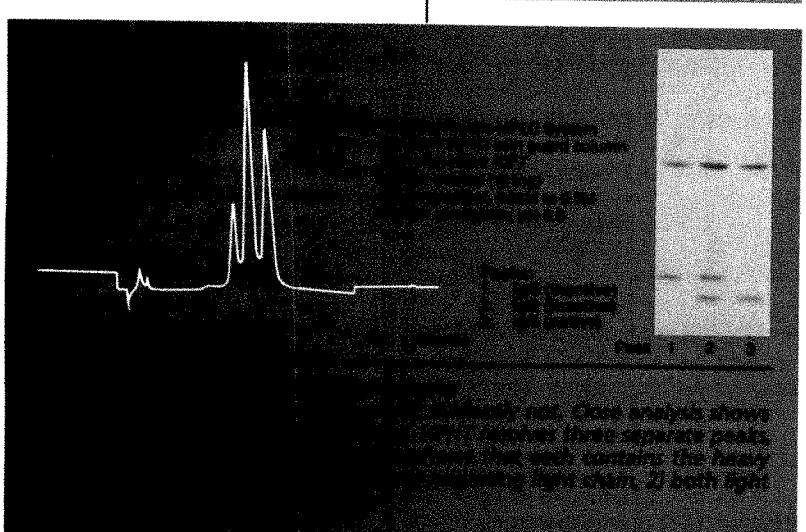
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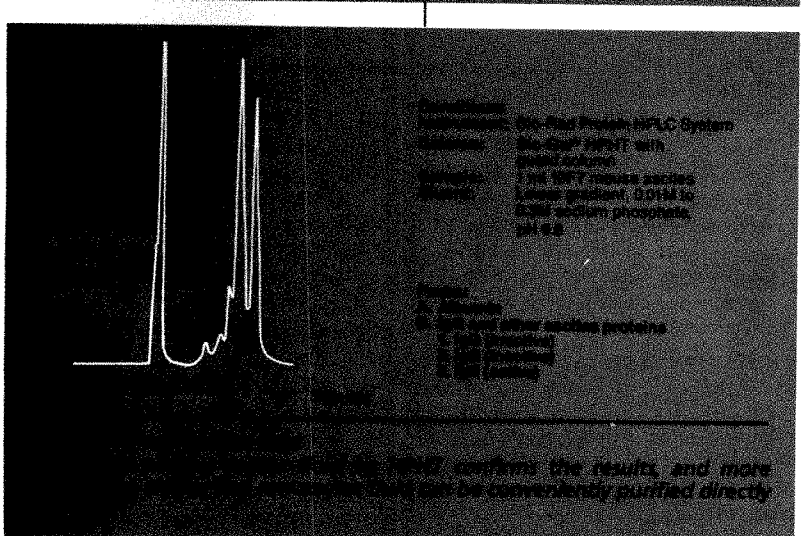
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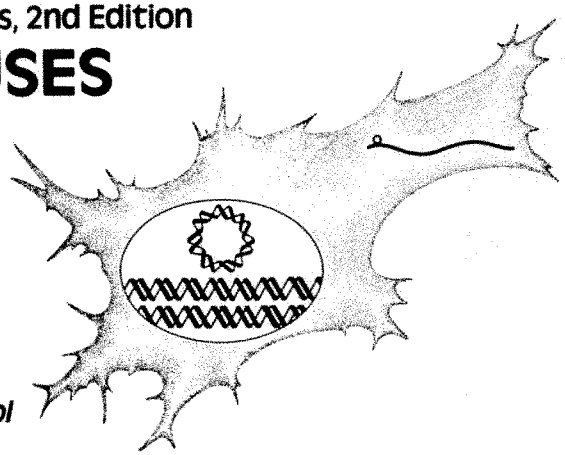
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Molecular Biology of Tumor Viruses, 2nd Edition

RNA TUMOR VIRUSES

Monograph 10C



Edited By
Robin A. Weiss, *Institute of Cancer Research, London*
Natalie M. Teich, *Imperial Cancer Research Fund Laboratories*
Harold E. Varmus, *University of California, San Francisco*
John M. Coffin, *Tufts University School of Medicine*

Since the original edition of *RNA TUMOR VIRUSES* first appeared two years ago, there has been an enormous increase in the amount of detailed information and a wealth of novel observations in retrovirology. Several progenitors of retroviral oncogenes have been implicated in human cancers, protein products of many oncogenes have been described, several new *v-onc* and *c-onc* genes have been identified, and sequencing of many retroviral genomes, LTRs, structural genes, and *v-onc* and *c-onc* genes has been completed. The result is a TWO-VOLUME PAPERBACK EDITION of *RNA Tumor Viruses* that is almost double the size of the original edition. Volume 1 contains the original text and remains the standard textbook for graduate students. The developments of the past two years, including a new chapter on human oncogenes, are recounted in Supplements to the original chapters. These appear in volume 2 along with new, expanded appendixes of extensive sequencing results and structural and restriction maps. Thus, those who already own the original edition need only purchase the second volume to update their hardcover copy.

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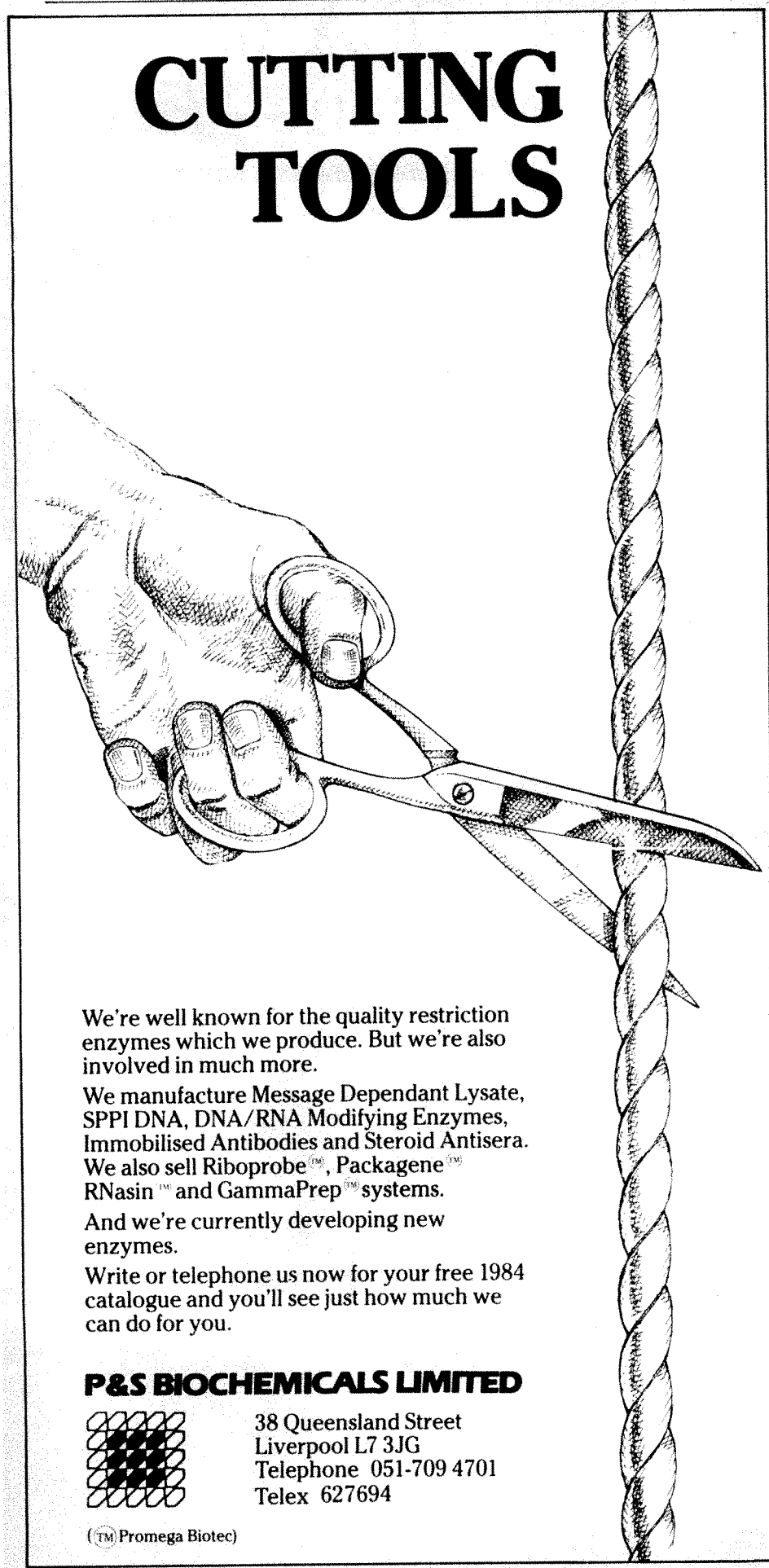


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nature

New machinery for UK science

After only a year, the new-style administration of British academic science has more enemies than friends. The best course would be to redesign the machinery, the best hope is that it will be improved.

THE British, humiliated earlier this week at the English national sport of cricket by a bunch of skilful Caribbeans, seem also to be heading for defeat in their next most gripping preoccupation, that of reorganizing the administration of publicly supported science. Although new arrangements for deciding how the budget for basic science should be distributed were introduced only at the beginning of 1983, the new system is already in trouble. The focus of general discontent is the Advisory Board for the Research Councils, the committee whose indispensable function is to tell the Department of Education and Science how the annual science budget for academic and basic research should be divided among its traditional recipients — five research councils, the Royal Society and the British Museum (Natural History).

For much of the time since the early 1970s, the board was a cosy committee of the executive heads of the research councils, the chief spenders of the science budget, accompanied by the chief scientists of other government departments. A select committee of the House of Lords then complained that the board had become too ingrown, too idle and too unreflective. The government declined to follow the advice then given that there should be a part-time minister with separate responsibility for civil science, but instead agreed that the board should be strengthened (which in practice has meant enlargement) and encouraged to take a wider view of the conduct of civil science. A year ago, those involved were full of hope that the new arrangement would function well. What has gone wrong?

Complaints abound, not the least of which is that the board has been turned into yet another talking shop of all the talents. Certainly it is too large to take decisions, or even to give advice decisively. The new chairman of the Economic and Social Research Council, Sir Douglas Hague, seems subversively to have been making this point to his colleagues in the past year, and cogently. To say the least, it is a sign of weakness that the board, for all its distinction, when faced with the question raised by the Science and Engineering Research Council whether the United Kingdom should continue to contribute towards the CERN high-energy physics laboratory, could not decide off its own bat but had instead to set up yet another committee, prolonging uncertainty by at least a year. Another difficulty that has arisen is that the research councils, whose chief executives remain members of the board, must turn themselves into supplicants of their lay (and less expertly informed) colleagues when they bid for their shares of the cake (most recently at the meeting on 7 June). It is galling to those concerned that the advice eventually given to the Secretary of State for Education and Science should be based on only second-hand knowledge of what the councils actually do with the money they are given to spend. The difficulty is especially acute when, as now, marginal changes in their budgets may determine the survival of particular laboratories.

Benefits

Before resentment of the present arrangements gets out of hand, its benefits should at least be acknowledged. For the past two years, the board has operated more publicly than previously, publishing both its advice on how the budget should be divided and (earlier this year) its sour response to the denial of extra funds. With luck (and time), it could yet become a means by which the British research community's sense of how limited resources should be used could be channelled more effectively into the conduct of public policy on civil science. The board has also, with its tiny and poor secretarial resources, commissioned some useful studies on questions such as the relationship between research institutes and universities, and between university research and industrial sponsorship. In passing, as part of its cake-cutting, the board has also pushed the research councils towards a greater concern for university research than for their own institutions. And while there is no prospect that, on present form, it will become collectively so knowledgeable about the pattern of British civil science as to refute the research councils' charge that it is inexpert, the need for such an all-knowing institution is itself questionable.

These frustrations would no doubt have been borne more easily in other, happier, times. If the British science budget were still growing every year, the great and the good would be able to supplement their advice to the Department of Education and Science with a few urbane comments on the opportunities lying ahead. Bad tempers have made their appearance only because those who spend the science budget, chiefly the research councils, are unable to satisfy the demands now being made on them by researchers seeking support for projects that are eminently worthwhile. It does not help that this year, the board has been compelled to repeat the demand, refused last year, for extra funds, with only committee-style eloquence to help. But these are precisely the circumstances — likely to persist for many years — when an effective commentary on public policy for science is needed. The strains that have become apparent are indeed a proof that the system needs to be changed. Here are three directions for reform.

Reform

First, most modestly, the present arrangements could be made to function more effectively. To that end, the board should be given an adequate secretariat, one composed of people (not necessarily civil servants) who understand the problems it needs to tackle. While the essence of the board's task may be to cut the annual cake of public spending on science, it could then hope to do this more intelligently by finding out what happens to the funds that eventually find their way into the hands of the research councils. Many obvious questions are unanswered. How many universities have set up research committees, as the board asked them two years ago, to plot a strategy for their institutions' research? (Some say that the answer is just one.) Are public research institutes collaborating more effectively with universities now that they have been bidden to do just that a dozen times? (Some are, some are not.) What is the effect on the pattern of academic research in Britain of the stream of exhortation by the British Government in favour of this, and then that, brave new opportunity? (Mostly baleful.) And is it true that, for reasons that are as much psychological as financial, the British research enterprise has lost its capacity to compete with others? (Probably yes.) What are the prospects — and problems — of strengthening inter-European collaboration in ways that would economize in effort, not waste it? (Many, but unexplored.) Without means of dealing intelligently with these and related questions, the board continues to lose conviction — and friends.

Second, and more imaginatively, the board could be given wider terms of reference. In the curious British way, its sphere of interest is confined to scientific research supported by the

Department of Education and Science. Other parts of the public research enterprise are within the purview of a different committee (the Advisory Council for Applied Research and Development), which lately has not surfaced in public view. There is no committee to ask questions about the relationship between civil and military research. It would still need a better secretariat. It could deliver the annual gobbet of advice to the Department of Education and Science more convincingly if it knew more directly what research is being sponsored by other departments. And it could avoid the present frustration by considering how the coordination between the disjoint halves of British public science could lead to greater effectiveness. In the real world, this is the most to hope for but the least for which to clamour.

Third, outrageously, is the remedy suggested in 1982 by the House of Lords: whatever apparatus there may be for giving specialized advice, let there be a minister specialized at receiving it. As things are, the British Government has built itself into a box. Lacking specialists within the civil service (as in West Germany) or an intimate relationship with the government's own specialist agencies (as in France), departments turn to advisory committees for advice which may be taken seriously when it is palatable and otherwise ignored. The downfall of the Advisory Board for the Research Councils can be traced to the occasion last year when it asked for more and was denied. Even if the Prime Minister rather than the Secretary of State was the one who finally said no, the doctrine of collective responsibility lumps the board in with all other dissident disappointed claimants on public funds — local authorities, schoolteachers and the unemployed. There is nobody to put the case, within the government, that the once and for all decline in the quality of the British research enterprise which is now on the cards will cast an even longer shadow. Within the political system to which the British are attached, but which allows ministers to carry only one torch at a time, only the designation of a single-minded political standard-bearer can enable the arguments for rescuing British science to be heard. □

Claims to antiquity

Publishers like to claim either antiquity or modernity. Cambridge can boast of both.

CAMBRIDGE University Press last week celebrated what it calls its 400th birthday with a "Garden Luncheon Party and Great Exhibition", offering its guests the presence of Prince Philip, the chancellor of the university, some sunshine, a band and a menu from which strawberries, usually mandatory on these occasions, were absent. Cambridge boasts that it is the oldest press in the world with an unbroken history. Only 80 years after Gutenberg, Henry VIII issued Letters Patent in 1534 granting the university "lawful and incontestable power to print there all manner of books". Although the first book did not appear until 1584 — even then, too long an interval to be explained as normal publication delay — it was a single but crucial year ahead of Oxford University Press and three years before the Vatican Press. Confusing for students of priority disputes was a 500-year celebration held by the University of Oxford in 1978. That occasion, Cambridge says, marked the quincentenary of the first book to be printed in the city of Oxford, not by Oxford University Press itself.

In science publishing, Cambridge University Press has been strong in mathematics and physics. In 1713, a revision of Newton's *Principia Mathematica* appeared under its imprint. Its other authors included James Clerk Maxwell, Rutherford, Jeans, Schrödinger, Einstein and, more recently, Hawking and Chandrasekhar. Undoubtedly the best known of the contemporary works is Joseph Needham's mammoth *Science and Civilisation in China,* Volume 6, Part 2 of which was published in April. Legally the press is unique, formally a department of the university with the status of a charity. It thus pays no tax. The press is governed by the 18 members of the syndicate, appointed by the university, which must approve all publishing proposals. Mercifully, this apparently anachronistic constitution seems not to be inhibiting. □

Unwelcome precision

Last week's US missile test could destroy the ABM treaty.

STAR wars are becoming uncomfortably realistic. That is one reading of the successful test last week of the US ground-based missile system, based on the Minuteman antiballistic missile, in which a dummy missile warhead was successfully intercepted within hours of a crucial decision by the US Senate about continuing support for these and related projects (see p.660). But last week's test was no part of the comprehensive defence against other people's ballistic missiles that President Reagan has been canvassing this past year. Instead, it is part of what some may call the conventional Anti-Ballistic Missile (ABM) programme which, in the United States and the Soviet Union, has its origins in the early 1960s. The fact that the test (the fourth) succeeded is nevertheless a sign that the treaties which are the fragile reminders of the time, not so long ago, when the superpowers felt able to sign agreements with each other may be coming to the ends of their useful lives.

In 1972, when the ABM treaty was signed between the Soviet Union and the United States, the openly acknowledged incentive was the fear that, without such a treaty, the deterrent value of strategic forces would be eroded. If one power believed that it could destroy the other's missiles, it would be less fearful of making a first strike with its own. That the two powers should thus openly acknowledge the identity of each other's calculations of the psychology of nuclear war was in itself a triumph. The treaty offered a little more — the promise of a respite from ruinously expensive development. But now those benefits appear to be receding. Both sides seem certain to be spurred by last week's test to further development. Technically, there is no reason why the new US system should not be stretched so as to become a second antisatellite (ASAT) weapon, capable of destroying satellites in low orbits, and thus a partner for the US Air Force system called MHV, launched from F-15 aircraft and already tested. (The traditional rivalry between the US Army and the Air Force will, left to itself, ensure rapid progress.) But technically, again, the arguments of the early 1970s remain valid that no rocket-borne system of this kind can be a certain defence against ballistic missiles with several warheads.

The first casualty will be the ABM treaty. The signatories accepted limitations on the testing of these weapons, although research was to be allowed. The signs now, however, are that unrestrained testing will create the impression, on one side or the other, that the treaty is a dead letter. Then, that is what it will become. And in such a setting, the prospects of an agreement on more elaborate systems to be deployed for longer periods above the atmosphere of the Earth will be even smaller than at present. The United States, after all, has not yet launched as many antisatellite weapons as has the Soviet Union, which began to test its system as long ago as 1968. President Reagan will be able to certify to Congress whenever he likes that he has complied with the Senate's qualification of its grant of star-wars funds by saying that no agreement with the Soviet Union would be "consistent with the national security interests" until the United States has done at least as well. But by then, the Soviet Union will have provided further goals at which to aim.

The depressing reality of all this is that it will be at the least a waste of resources on both sides, and will be dangerous as well. While the star-wars idea is said (by some of those privy to what is planned) to be a technically interesting bag of innovations, nobody has been able to convince the sceptics that a star-wars defensive system could destroy a high proportion of the other side's missiles. And for as long as that remains the case, the course on which the Senate has allowed the US Administration to embark, and on which the Soviet Union is already engaged, cannot counter the objections raised against ABM defence in 1972: if there is a nuclear balance, it is bound to be upset. The irony, of course, is that the two governments were prepared to accept the logic in 1972. They should now follow suit for star wars. □

Australian uranium

Chance of policy change on nuclear fuel

Canberra

THE Australian Labor Government has been given an influential blessing for a change of policy on uranium exports. On 31 May, the Prime Minister, Mr R.J. (Bob) Hawke, presented to the federal parliament the report of a committee which argues that the mining and milling of uranium should continue and that the government should have authority to approve new mines and supply contracts.

If eventually adopted, the policy now recommended will represent a substantial change in the policy which the Labor Government has followed since its election. During the past year, the federal government has allowed existing operations and contracts to continue but has set its face against new enterprises. The new report argues not merely that the supply of uranium overseas should be increased but that Australia should participate in the enrichment of uranium, the reprocessing of nuclear fuel and, possibly, other steps in the nuclear fuel cycle.

These recommendations will strengthen Mr Hawke's hand in influencing uncommitted delegates to the Australian Labor Party (ALP) national conference next month, when strong opposition from the left is expected to the government's policy of allowing existing mining to continue, even under stringent supply conditions.

The 300-page report, *Australia's role in the nuclear fuel-cycle*, was prepared at Mr Hawke's request by the Australian Science and Technology Council (ASTEC), under the supervision of its chairman, Professor Ralph Slatyer, former Australian Ambassador to the United Nations Education, Scientific and Cultural Organization, when in November last year the government granted permission for the development of a new uranium mine at Roxby Downs, South Australia, potentially the world's largest.

The Slatyer report recommends that Australia, although not itself likely to use nuclear power, should accept that for many countries nuclear energy has become a fact of life. And since Australia owns 30 per cent of the uranium resources of the West, its contribution to nuclear non-proliferation should be enhanced by participation in the nuclear fuel cycle.

As a reliable long-term supplier of uranium, Australia would be in a position to influence the behaviour of other states, the report argues. Thus, by helping to ensure international energy security, Australia might reduce the motivation of other countries to engage in other steps of the fuel cycle, particularly reprocessing and fast-breeder development.

The compilers of the report are unconvinced that supplying uranium to nuclear weapons states frees other uranium for use in weapons (vertical proliferation). Denial of supply to such states, the report says, would be an empty gesture, scarcely affecting weapons programmes, since stockpiles are already adequate. Similarly, denial would not deter horizontal proliferation, since the political decision to develop nuclear weapons can in present circumstances be implemented by any country with sufficient determination and a reasonable scientific infrastructure. The absence of a civil nuclear industry would not prevent the manufacturer of nuclear weapons. In any case, the report adds, such a country is unlikely on efficiency grounds to rely on civil power reactors for the manufacture of plutonium.

Other recommendations include continued support for a nuclear weapons-free zone in the South Pacific and for an Indian Ocean zone of peace. Australia should seek to join the board of governors of the International Atomic Energy Agency and spell out the feasibility of regional treaties with South and East Asian countries along the lines of the Treaty of Tlatlelco. The regime should be strengthened by internationalizing the steps in the fuel cycle, but sensitive facilities, particularly enrichment and reprocessing plants, should be located in as few countries in the region as possible, Australia and Japan for example. There should be support for the moratorium on ocean dumping of low-level waste, and continued research on the Australian-designed ceramic Synroc, the most promising high-level waste form, but Australia should not be obliged to dispose of high-level wastes from Australian uranium.

For the government and Mr Hawke, everything now hangs on the outcome of the Australian Labor Party's national conference, which seems unlikely to endorse a uranium policy going beyond that now defined by the government party caucus at Canberra and which is opposed to further uranium mines after Roxby. A further difficulty for the government stems from the ban on uranium sales to France, intended as a protest against French nuclear tests in the Pacific, the latest of which took place on 17 June. Unless the tests end, the Labor Party is unlikely to relax its traditional view that Australian uranium should not be used for other people's bombs. This, the government fears, may lead to trade reprisals and even to a law suit at the International Court of Justice.

Jeffrey Sellar

US–China nuclear treaty

Congress makes difficulties

THE United States–China nuclear exchange agreement which President Reagan announced triumphantly in Beijing last April is almost certain to be postponed and may fall through altogether. The text of the agreement initialled in Beijing has not yet been submitted for congressional approval, and the administration is now conceding privately that the assurances on non-proliferation may not be explicit enough to satisfy Congress.

A State Department spokesman said last week that the text had been studied by the relevant federal departments, including the Nuclear Regulatory Commission, and found to comply with US law on the transfer of nuclear technology. But he also revealed for the first time that the administration had sought renewed talks with the Chinese Government "to ensure a full mutual understanding".

What appears to have happened is that the administration was so eager to close the deal while President Reagan was still in Beijing that it fudged the two issues that had already dragged out the negotiations for more than two years. One is the status of China's promise not to help other nations to acquire nuclear weapons; the other is a requirement that China must seek US approval before using nuclear fuel from American-supplied reactors in nuclear warheads or for warship propulsion.

Persistent allegations in Congress that China has assisted Pakistan in a nuclear weapons programme have been a major reason for Congress's lukewarm attitude. Last week, however, the *Washington Post* published a startling news story maintaining that the US delegation in Beijing had decided to accept verbal assurances on non-proliferation from Premier Zhao Ziyang instead of insisting on explicit written assurances in the text of the agreement. There had previously been reports that the thorny problem of China having to ask for US permission to use reprocessed fuel from US-supplied reactors had been overcome by "clever drafting".

Just why the administration has had second thoughts about the adequacy of the safeguards it claimed to have negotiated successfully last April is a mystery. Officially-inspired leaks suggest that new intelligence information has confirmed China's role in Pakistan's nuclear weapons programme.

It is just as likely that the Nuclear Regulatory Commission and other agencies with close experience of Congress's views on nuclear proliferation have concluded that the agreement is too vague to be approved in its present form.

Peter David

Electron accelerators

Southern US project delayed

Washington

THE group of US universities unexpectedly selected last year to build a major new electron accelerator has begun hesitantly. After a year, the project still has no director and, in the absence of a definite estimate of construction costs, Congress is balking at appropriating the full amount requested by the Department of Energy (DoE) for the project, now known as the Continuous Electron Beam Accelerator Facility.

The Southeastern Universities Research Association (SURA), a consortium led by the University of Virginia, was chosen last year by the Nuclear Science Advisory Committee (NSAC) to build the new machine at a site in Newport News, Virginia.

The decision set off a long political battle when Argonne National Laboratory, which had hoped to win the committee's recommendation, took its case directly to Congress and the Secretary of Energy. Traditionally, the physics research community has accepted as final the decisions of NSAC, which is made up of nuclear physicists and is formally an advisory body to DoE.

The fight between Argonne and SURA may have played a part in the slow start of the project. James McCarthy, a professor of physics at Virginia who is acting director of the accelerator, said that last year's fight gave the project a "political visibility" which provided greater congressional scrutiny, even after the Secretary of Energy had settled the matter last summer in SURA's favour.

The House of Representatives approved the administration request of $5 million for research and development and $2 million for construction in fiscal year 1985, but the Senate, complaining about not having a detailed cost estimate or a clear justification for the project, cut the figures to £2.5 million and zero respectively. At the bidding of Senator Bennett Johnston, the senior Democrat on the Senate Energy Committee, the Senate also asked DoE to provide a five-year plan for nuclear physics that would include the options of going ahead with the SURA accelerator or dropping it.

Although SURA would still be allowed to go ahead with the detailed architectural and engineering design work it had called "construction" in its budget request, the Senate's elimination of construction funds serves notice that the future of the project is by no means certain. It is generally difficult to kill a project once it has been accorded a line-item construction authorization.

SURA originally proposed a budget of $20 million for next year, but backed off in the face of congressional complaints that the ultimate cost is uncertain. SURA's original proposal was that the total cost would be about $100 million, but NSAC, considering that unrealistic, increased it to $150 million. In testimony to Congress this spring, McCarthy mentioned a figure of $225 million. Although most of the increase can be explained by inflation, there appeared to be some new items (such as increased office space).

Some of the Senate's caution may stem also from worries about whether the United States can afford such projects if it is to go ahead with the massive proposed Superconducting Super-Collider, which may cost $3,000 million.

D. Allan Bromley of Yale University, who chaired the NSAC panel that recommended SURA, said that the physics community still considers the project to be a first priority. He said that although there is widespread disappointment that more progress had not been made, much of the trouble is a result of a "Catch-22" dilemma; SURA cannot attract a director without assured construction funds, and cannot get assured construction funds until it has a director and a definite design.

According to McCarthy, an offer has been made to a "leading accelerator physicist", who will decide by 1 July whether to accept the directorship. That date is also when SURA will receive $1 million appropriated by the Virginia legislature and an additional $1 million that Congress has just allocated to DoE to "reprogram" the project. The funds will allow SURA to begin filling 9 senior and 15 junior positions on the scientific staff.

Stephen Budiansky

Computer theft boom

Washington

A SURVEY of US companies and government agencies has provided the first systematic evidence of how widespread computer crime has become. Of the 283 organizations that responded, 72 reported "known and verifiable" losses as a result of computer crime in the past 12 months. The average loss was in the range of $2–10 million, but one organization reported losing more than $100 million.

The survey, conducted by the American Bar Association, defined "computer crime" quite broadly to include embezzlement, theft of software and even the unauthorized use of computers for personal programming. The last was found to be the most common "crime", reported by 62 respondents; next in line was the theft of software, reported by 45, and use of computers for theft of assets (44).

While it is impossible to extrapolate the survey's results, the bar association states that "given the small number of organizations reporting these large losses . . . the total loss figure nationwide would appear to be enormous." The survey was sent to 1,000 organizations Two-thirds of the identified perpetrators were employees of the organization, most often computer programmers.

The bar association called for the enactment of federal legislation against computer crime to aid in the reporting and prosecution of such incidents. Existing statutes that may be applicable, such as wire fraud, trespassing and embezzlement, carry penalties that the association said are often "far from commensurate" with the seriousness of computer crimes.

Stephen Budiansky

Academic ethics

Perils of scholarly plagiarism

Palo Alto

A distinguished Stanford University professor resigned as chairman of the department of medicine last week after a university ethics committee found him guilty of "grossly negligent scholarship". Dr Kenneth Melmon, who keeps his faculty position, was censured for plagiarizing roughly a quarter of a chapter in an endocrinology textbook. The committee said that no fraud had been committed and that Dr Melmon had had no conscious intent to deceive.

Trouble arose when the publisher of another medical text, *The Pharmacological Basis for Therapeutics*, found that large portions of that had appeared in Dr Melmon's chapter contributed to the endocrinology book (see *Nature* 5 April, p.489). At the time, Dr Melmon said his friend and editor of the endocrinology book, Dr Robert Williams, had urged him to use portions from the pharmacology book, promising that permission and attributions would be secured. Dr Williams died, however, while the endocrinology book was being edited and his personal papers were later destroyed.

The Stanford ethics committee now says that it accepts Dr Melmon's explanation but has nevertheless censured him for his "recklessness" and "carelessness in dealing with the intellectual properties of others". He violated "the fundamental norms of the academic profession".

Dr Melmon was responsible for ensuring that proper attribution was incorporated into the chapter, the committee says; he should have checked that it was done properly.

Dr Melmon said he was resigning his chairmanship because "the mistake was a serious one". A clinical pharmacologist, he holds the Bloomfield endowed chair in Stanford's department of medicine and is a member of the Institute of Medicine of the National Academy of Sciences.

Sandra Blakeslee

Japan's unemployed PhDs

Unused researchers to arms

Tokyo

JAPAN's ever-increasing numbers of unemployed postdoctoral researchers are banding together to find ways of making life a little easier. But there is little they can do to increase their chances of actually finding an academic job.

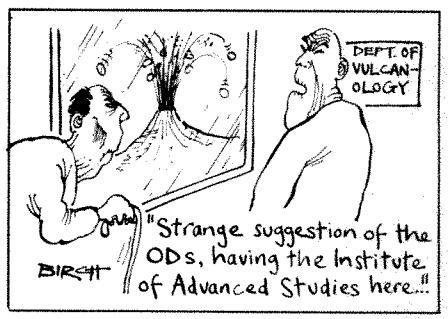
The number of "overdoctors", or "ODs" as unemployed postdoctoral researchers are known in Japan, is now at an all-time high. (Overdoctor is an apt English word which the Japanese have invented for themselves.) There are at least 5,000 waiting for jobs in university departments, many of whom have now been waiting for more than ten years.

Japanese universities, unlike many of their Western counterparts, officially allow students who have completed their doctorates to stay on in their laboratories (upon payment of a monthly fee of around £40) to continue unpaid research while looking for a job. In some cases, such as the physics department of Kyoto University where there are now over a hundred, they outnumber the full-time teaching staff. Physicists, particularly theoretical physicists, have been hardest hit, with more than a thousand ODs nationwide, followed by humanities, social science and agriculture.

To ease their plight, Dr Teruhisa Komatsu, an oceanographer from Kyoto University, has set up an OD Society and has found himself 200 members in just a few weeks. The society's chief aim is to keep ODs going, both financially and psychologically, while they are looking for *arubaito* (part-time work, this word stolen from the German *arbeit*) — usually teaching in a cramming school, translating technical reports from European languages or helping with the sophisticated analysis of university entrance requirements needed to help high-school students to pick a suitable university. The society plans to help find the best jobs for its members and also to remove the ODs' sense of isolation by arranging conferences for the presentation of their work.

Life as an OD is by no means easy. Although most ODs are in their late twenties, surveys show that only a little over half are married (compared with nine-tenths of the general population of the same age). Average incomes are exceptionally low, only a little over £3,000 a year before tax.

A particularly cruel irony faces those who never find academic jobs. During their postgraduate study (two years for a master's course followed by three years of doctoral research), students must pay their own fees of around £400 a month as well as their own living expenses. There are no cosy European-style grants to cover the cost of education. As a result, almost all students take loans from semi-governmental bodies. While the loans need not be repaid by those who find a university job, the unemployed are left with a huge bill for their education.



Neither the Ministry of Education, Culture and Science (MESC) nor university staff are taking steps to ease the OD problem, mainly because everyone lays the blame at someone else's door. One ministry official sums it up by saying that "they (the ODs) should seek work in practical, non-academic fields, instead of keeping their sights on a life in the ivory towers".

Certainly it is a common view that ODs bring about their own problems by "refusing" to work in industry, insisting instead on waiting for the impossible. There is some truth in this charge. Among physicists, for example, there are many who have had experience of computer programming and are in demand by industry. But it is nonsense to suggest that most ODs can easily find work of any other kind than the part-time work they already do.

Industry in Japan has a strong preference for graduates fresh from university, who can be trained in a company's own ethos. Even school teaching is hard to enter, with an upper age-limit for new entrants of around 28–32. Particularly hard-hit are the many ODs with training in agriculture, for there is no related practical field into which they can go. Small wonder, then, that the two most favoured routes out of the OD trap are a fellowship to a US or European university — and journalism.

The only long-term Japanese solution must be an expansion of the universities, but this is a political question. And the government intends to squeeze all the ministries again next year (with the exception of defence). Hopes of university expansion must for the time being remain in the realms of fantasy. **Alun Anderson**

Academic demography awry

THE OD problem results from the massive expansion of the universities in the late 1960s, which created many new doctoral schools. With the oil shock, the expansion of the universities has been halted, but there are fewer job opportunities for researchers from the new schools.

So why cannot the Ministry of Education, Science and Culture (MESC) reduce the flow of graduate students, or at least redirect them into areas, such as computer programming, where highly-trained people are needed? Unfortunately, the Japanese system is far from flexible. Once permission has been gained for the establishment of a doctoral school, there is little further MESC can do to control it. As no grants are given directly to students (and loans are easy to obtain), it is hard to redirect students quickly into new subject areas. And professors, enjoying considerable power under the "chair" system, will fight tooth and nail to prevent government meddling in their affairs.

Thus arises a common view within MESC that professors worried about the OD problem should voluntarily reduce their student intake and coordinate activities with others in their field to take account of future forecasts. Some professors have indeed done exactly this. But because teaching loads are high, it is virtually impossible for any ambitious professor to carry out a research programme without graduate students to help.

The contraction in the universities that has provoked the OD problem has also caused another, potentially more damaging, academic problem, one which MESC seems again to be ignoring. The slowdown in the recruitment of new researchers has seriously distorted the age structure in the university research community. Simulations conducted by Kenichi and Kaoru Aoki of the Kyoto University Research Institute for Fundamental Physics show that, by 1991, the median age of university staff will peak very sharply at 48. By 2001, the distribution will be bimodal with the larger peak at age 58.

European countries, particularly West Germany, are no strangers to this problem. The difference is that in Europe studies are being made by official bodies rather than being left to private individuals and steps are being taken — such as the United Kingdom's "new blood" scheme — to ensure that there are enough young workers at universities.

Drs Aoki hold that Japan just does not take a long-term view of scientific research planning — day-to-day economics are the prime determinant.

Postdoctoral fellowships, at present non-existent in Japan, could bridge the immediate shortage of young university workers until retirements begin to ease the situation. The Aokis claim that with such fellowships, an average expansion of 1,800 university posts a year, well below the 1960s peaks when 5,000 new jobs a year were being created, would give the universities a very healthy age structure. Alun Anderson

Weapons in space

ABM test persuades US Senate

Washington

LAST week's interception of one Minuteman missile by another at an altitude of more than 100 miles was a feat of exquisite political timing. The successful test was announced by the Department of Defense at the beginning of a week in which the Reagan Administration's plans for testing a new antisatellite (ASAT) weapon and stepping up research on the proposed "star wars" nuclear defence system faced major congressional hurdles. In the event, the Republican-controlled Senate agreed to give the ASAT test an amber light. And Congress now seems certain to give the administration much if not all of the $2,000 million it wants in 1985 for research on ballistic missile defence.

Of the two space weapon initiatives, the more pressing from the administration's point of view is the ASAT test. The Air Force has already developed a sophisticated aircraft-borne satellite interceptor (see *Nature* **308**, 576; 1984), but the House of Representatives voted last month to block tests against targets in space for a year or for as long as the Soviet Union continued a moratorium on tests of its own weapons. In both houses, there is strong feeling that talks on an ASAT ban should be resumed, despite the administration's insistence that no such ban could be adequately verified. And in a piece of deft timing of his own, Soviet President Konstantin Chernenko again urged the United States to negotiate an ASAT ban before it was "too late".

To discourage the Senate from following the example of the House, President Reagan's Senate allies arranged a closed session in which senators received intelligence briefings on the state of Soviet ASAT technology. They are said to have been told that, in addition to its well-publicized ASAT interceptor, the Soviet Union now has the means to cripple US satellites with a laser beam from an Earth station or by jamming their radio communications.

A day later, the Senate voted by 61 votes to 28 to allow the ASAT tests to proceed provided only that the President shows that he is trying to negotiate "the strictest possible limitations on antisatellite weapons consistent with national security interests of the United States".

The effect of the Senate resolution, which will have to be reconciled with the position of the House in a conference committee, is to require some action by the administration that demonstrates a wish to prevent an arms race in space. But it is clear from the mood of the debate that a symbolic gesture will suffice. As long as Mr Reagan certifies that the ASAT tests are vital to national security, and will not represent an "irreversible" block to negotiation, he will be allowed to test the new weapon.

Whether the President will take up the option in a hurry is another matter. The administration is becoming uncomfortably aware of growing public concern about the prospect of an arms race in space. So is the Democratic Party; its national chairman, Mr Charles Manatt, said last week that the presidential election in November will give voters a chance to ponder the "frightening choices" entailed in the administration's plans for the militarization of space. President Reagan is under pressure from his own party to show some flexibility on arms control before the election; by the end of last week the administration was already hinting that it might be willing to discuss some limitations on ASAT development.

The short-term ASAT question has not entirely distracted attention from "star wars". While Congress appears still to be willing to underwrite the preliminary research called for in the 1985 budget, organized opposition to the concept is gathering pace. A "national campaign to save the antiballistic missile (ABM) treaty" was launched this week by a committee of senior politicians and scientists, including former President Jimmy Carter and former defence secretary, Robert McNamara. In an opening statement, the committee claimed that the expanded star wars testing programme requested by the administration will "soon collide" with the restrictions imposed by the ABM Treaty.

Meanwhile, in a bizarre coda to the political debate, the technical debate over the feasibility of the star wars programme is becoming increasingly unfriendly. Deputy Defense Secretary Howard Taft has taken the extraordinary step of asking the Office of Technology Assessment to withdraw a technical memorandum on the subject written by Dr Ashton Carter of the Massachusetts Institute of Technology (see *Nature* **309**, 485; 1984). Mr Taft claims that the memorandum, which said the prospect of a near-perfect defence against nuclear attack was "remote", had subsequently been criticized on technical grounds by four independent review groups.

The Pentagon clearly expects last week's Minuteman interception to cut some ground from under the feet of those who doubt whether a defence against ballistic missiles is feasible. However, most critics of the star wars concept have already acknowledged that the interception of nuclear warheads in midcourse is feasible. Their objection is that a nuclear attack would involve so many missiles and decoys that such defences would be overwhelmed. The Office of Technology Assessment study therefore focused on the prospects for destroying missiles in their boost phase, before they could spawn their mutiple warheads. It concluded that most of the novel technologies required could be countered with relative ease.

Peter David

US Naval Observatory

Research at risk

Washington

THE US Naval Observatory, one of the oldest government scientific institutions in the United States, is in trouble, according to the National Academy of Sciences. Short of funds and overburdened by its role as a support agency, the observatory's basic research capability is in jeopardy, according to a new study. Some divisions of the observatory are "one man deep", according to Lee Hunt of the National Academy staff, and are "struggling to provide the bare minimum of services". Scientists often have to act as administrators and technicians as well, all of which leaves little time for basic research.

The primary mission of the observatory is what it always has been: providing data to aid navigation. Much of its work at present, for example, involves calibrating



atomic time standards and carrying out the observations needed to adjust the International Atomic Time scale for astronomical and navigational use.

But the observatory has also established a solid scientific reputation. The study notes the observatory's longstanding work on visual double stars, now in jeopardy for lack of manpower, and its determinations of parallaxes of faint stars — data that "effectively define our knowledge about luminosities, colours and tangential velocities of faint stars in the neighbourhood of the Sun". "Too much of the astronomers' time must go toward technical support activities", the study says, but Hunt put the point more directly: "You find directors sweeping floors."

The shortage of manpower is apparent in the study's recommendation on the double-star programme. If "one other person were able to take over clerical jobs", the study says, "the staff would be able to do much needed work on new technology." Hunt said that the study panel felt that continued excellence in basic research was vital to attract qualified scientists and to keep them in the mainstream of science.

The observatory has suffered not so much from budget cuts as from benign neglect. But the consequences are just as serious, the academy panel said. "No action at this time is a decision to erode the quality of staff and the services of data and information it provides."

Stephen Budiansky

UK medical research

More MRC units threatened

THE parlous financial position of Britain's Medical Research Council (MRC) is obliging the council to reconsider the future of any research to which it is not "irrevocably committed". The futures of three research units supported by the council are now described as "under review" following critical reports by visiting parties that, in some cases at least, recommended the council to consider closure.

The council said that a decision had been taken "in principle" to close its unit of neurochemical pharmacology at Cambridge (see *Nature* 7 June, p.486). But, as if to advertise that British medical research is not completely moribund, the council has also announced a new research unit to undertake laboratory studies of tuberculosis.

Surprisingly, the location of the new unit has not yet been finally decided, although the probable choice will be the Royal Postgraduate Medical School at Hammersmith, London. The new unit will replace two existing units in London, one at Hammersmith and one at the Brompton Hospital, Fulham, which are to be closed when their directors retire shortly.

The formation of the new unit signals a shift away from the study of the effects of tuberculosis on communities and of chemotherapy towards the use of new techniques, including recombinant DNA technology and monoclonal antibodies. The director is to be Dr Juraj Ivanyi, now head of experimental immunobiology at Wellcome Research Laboratories.

The three units whose future is uncertain are the Biostatistics Unit at Cambridge, the Pneumoconiosis Unit at Llandough Hospital in Penarth, South Wales, and the Mineral Metabolism Unit based in Leeds. A report by a regular visiting group to the Pneumoconiosis Unit has recommended closure, and the matter is to be considered shortly by MRC's Physiological Systems and Disorders Board. There has been a cloud over the future of the unit for some time, but the recommendation to close it was still a surprise to staff, who say they will be able to enter pleas for continuation when the board's recommendations, together with the unit's responses, are formally considered by MRC. The unit has no director in post at present, thus facilitating any closure decision.

The Mineral Metabolism Unit is also without a director, but its acting director, Dr M. Peacock, seems unwilling to accept that closure is one option to be considered by the disorders board. The unit's work on osteoporosis and adrenal stone disease is in line with recommendations of an international review of calcium in metabolism, and Dr Peacock is confident that MRC will find "no scientific grounds" to close the unit, although there have been some recent staff reductions. Much the same applies to the Biostatistics Unit, where interviews for a replacement director for Dr I. Sutherland have been called off at short notice.

Tim Beardsley

No sale yet for UK chip-maker Inmos

THE British Government is apparently still undecided about how it should introduce private capital into Inmos, the state-backed semiconductor manufacturer. The company, which started to trade profitably in the final quarter of 1983, has been provided with government capital of £65 million and loans and guarantees worth £40 million, and the Secretary of State for Trade and Industry, Mr Norman Tebbit, is keen to reduce the strain on the public sector borrowing requirement.

Earlier this month, Mr Kenneth Baker, Minister for Information Technology, said in the House of Commons that the government had vetoed a private share placing organized by the company that would have raised £30 million, on the grounds that the terms were not sufficiently attractive. Mr Baker's answers to supplementary questions made it clear that the government is anxious that Inmos technology should continue to be accessible to British industry and that there should be a commitment to continued development and expansion of Inmos's activities in Britain.

More recently, Thorn EMI has made public its offer of £10 million for a share of the company, an offer which Inmos's chairman Sir Malcolm Wilcox described as "very interesting". But the matter is still under consideration by the Department of Trade and Industry, which oversees the nominal holder of public shares in Inmos, the British Technology Group.

The US communications group AT&T (American Telephone and Telegraph) is also now bidding again for Inmos. An approach by AT&T was rejected by the board of Inmos in February, but negotiations are now under way directly with the government. AT&T has, as a nod towards the principle of keeping some of Inmos's activity based in Britain, agreed that some of its research and development activity might be carried out by ICL, the British computer company. AT&T is understood to have made an offer of £45 million for the whole company, a figure regarded as derisory by Inmos management.

Inmos is now making a monthly profit of around £1 million and has great hopes for the future with its revolutionary "transputer" chip. Tim Beardsley

Neural tube defects

British vitamin trial starts slowly

THE controversial British clinical trials of vitamin supplements in the prevention of spina bifida and other neural tube defects has begun, but on a much smaller scale than originally intended. A progress report issued last week said that so far the Medical Research Council (MRC) has recruited 132 women to the trial, still far short of the 2,000 target.

Even so, MRC says it is reasonably confident that recruitment will accelerate as new centres join the study. So far, physicians at a total of 16 medical centres in the United Kingdom and 12 overseas have agreed to take part, all with the approval of their local ethical committees.

The decision to conduct control trials with vitamin supplements was taken in December 1982, partly because of research by Professor R.W. Smithells at the University of Leeds which seemed to show that neural tube defects might be prevented by vitamin and particularly folic acid supplements before and immediately after conception. To separate the effects of folic acid from those of other vitamins, MRC advocated clinically controlled trials with randomly selected patients.

The trial has been controversial because the four groups of women to which volunteers are assigned at random includes one to whom only a placebo will be administered. During the past two years there have been complaints that the use of a placebo group is unethical in the face of *prima facie* evidence that vitamin supplements will prevent neural tube defects, and that it may even be a breach of the revised Helsinki code of 1975 on the use of human subjects in research. There is also some concern that the daily dose of folic acid being administered to one of the four groups in the MRC study is, at 4 milligrams per day, ten times that used in earlier work.

Recruits to the study, who must first declare themselves to their personal physicians, include only women who have already given birth to a child with spina bifida or encephaly and among whom the chance of a recurring defect is, at 3 per cent, comparatively high. MRC insists that the anxieties of protesters such as the National Childbirth Trust at the ethical basis of the trial are answered by its information leaflet for participants, which says that the consent of the woman's husband will normally be required and that women's medical care will not be diminished if they do not volunteer.

So far, 18 of the 132 recruits to the study have become pregnant (and one has produced a normal child). Whether the target number of 2,000 recruits will be found within five years is at this stage by no means certain. **Judith Abel**

Artificial reproduction

Unrestrictive guidelines for UK

THE Warnock Committee's recommendations to the UK Department of Health on artificial reproduction began accurately to leak out last week. The main conclusions of the 18-month study, due to be published at the end of June, are that commercial surrogate motherhood agencies should be banned; that hospitals and clinics specializing in *in vitro* fertilization (IVF) and artificial insemination by donor (AID) should be licensed and required to register successful births; that the children born through AID programmes should be legitimized; and that research on embryos should be limited to 14 days after fertilization.

To administer these provisions, the committee recommends a permanent national licensing body whose role would also include monitoring embryonic research and advising government on new developments in artificial reproduction techniques.

On the issue of AID births (already accounting for at least 2,000 children in Britain each year), it has also been recommended that no sperm donor should be allowed to father more than ten children and that his identity should remain undisclosed; it is not yet clear, however, what subsequent access a child conceived by the AID method would have to information about his or her natural father.

The broad conclusions are not, however, unanimous. Two of the 16 members of the committee hold that surrogate motherhood should not be completely banned, but accessible only when there is no other option for a childless couple. The dissenters advocate that, in these circumstances, arrangements should be undertaken by non-profit-making bodies such as adoption and fostering agencies; the British Agency for Adoption and Fostering is not, however, enthusiastic about the suggestion.

On embryo research, two further minority reports (signed respectively by 3 and 4 members of the committee) are expected; one opposes on principle all research on human embryos, the other argues against the special creation of embryos for research purposes.

First indications are that the committee's report will be fairly well received by physicians and researchers, although some claim that the 14-day limit on work with embryos is too restrictive. (The Royal College of Obstetricians and Gynaecologists has recommended a 17-day limit.) The committee also eschewed a time limit on how long embryos can be kept frozen where parents desire several children, although the general specification is that it should be long enough to allow for children to be well spaced. Parents should be consulted on the fate of spare embryos, the committee says.

The leaking of the Warnock Committee's recommendations has been greeted with relief both by researchers fearing restrictive regulations and by those who see a need for swift legislation, especially with increasing commercial interest in surrogate motherhood.

Despite the sense of urgency in the scientific community and, indeed, among the Warnock Committee members, government legislation is not likely before October 1985. But there is likely to be mounting pressure on politicians to achieve some form of legal guidelines well before then, especially once the report is published, later this summer. **Judith Abel**

British universities

Another turn of the screw

WHILE British universities contemplate the news that their recurrent grant is likely to fall in value by more than 2 per cent over each of the next 2 years, six of them have been named as guinea pigs for a study of university efficiency being undertaken by the Committee of Vice-Chancellors and Principals (CVCP) and the University Grants Committee. The chosen six are the Universities of Edinburgh, Essex, London (University College), Loughborough, Nottingham and Sheffield. These institutions will during the coming autumn collaborate in efficiency studies with a steering committee under Sir Alex Jarratt.

Although universities have been anxious to appear willing to cooperate in such efficiency exercises, their temper has not been improved by recent estimates circulated by CVCP which indicate that the recurrent grant is likely to fall in value by 4.4 per cent between the present financial year and 1986–87. The estimates are based on "indications" provided by the University Grants Committee and were arrived at by excluding the protected Information Technology and New Blood schemes and then incorporating government expectations for inflation over the next 2 years.

CVCP's document, circulated to vice-chancellors at the beginning of this month, argues pointedly that provisional levels of recurrent grant incorporate two levels of reduction in value: scheduled cash increases are lower than projected cost increases, and the cost increases are themselves lower than government expectations for inflation. The cash value of the recurrent grant is expected to rise from its current level of £1,249 million to only £1,320 million in 1986–87.

Mr Michael Baatz, secretary of the efficiency study group, emphasizes that the initiative for efficiency studies came from CVCP itself, albeit "in response to government interest". All British universities had declared themselves willing to take part, but the chosen six represent a variety of university types, old and new, large and small. The study is supported by a grant of £300,000 from the Department of Education and Science.

The plan for the study now agreed is unusual in at least two respects. Eight topics have been drawn up as suitable for investigation, ranging from the all-embracing ("financial management") to the particular ("animal houses"). But only one topic will be investigated at each of the six universities included in the study (the topic to be chosen jointly by the university and the committee), and so at least two of the possible topics will be left untouched. And the committee is pledged to make no judgement on academic matters, such as what subjects should be taught.

Mr Baatz admits that there will be much discussion on what constitutes an "academic" matter. While questions such as level of provision for each subject are clearly out of bounds, the committee will hope to make recommendations on how resources might best be deployed to achieve stated aims. The academic side of these questions is being tackled by a separate CVCP working group on academic standards chaired by Professor Philip Reynolds, vice-chancellor of the University of Lancaster. **Tim Beardsley**

Reagan's Lysenkoism

US PRESIDENT Ronald Reagan should be awarded a special "Lysenko prize" for his "star wars" theories, Academician Evgenii Velikhov asserted last week. Velikhov, a vice-president of the Soviet Academy of Sciences and chairman of the Committee of Soviet Scientists for Peace and Against Nuclear Wars, was speaking on Moscow Radio's "English for North America" service.

According to Velikhov, Reagan's allusion to "new defensive systems" that would give the United States an antimissile capability was simply "Lysenkoism". Present-day lasers, he said, would have to be improved by ten million times to provide a reliable antimissile defence system. Like Lysenko, he says, Reagan is prepared to "put science into storage" if it gets in the way of his theories.

The hope of "protecting all people" is a "wonderful concept", but, like Lysenko's agricultural theories, is in reality "a lie" which cannot be implemented.

Velikhov says he made this clear during a visit to the United States last month, in meetings with the Federation of American Scientists, the US National Academy of Sciences and "people with political and military experience". Vera Rich

Baikal-Amur railway

Building too fast for efficiency

SOVIET plans to complete construction of the Baikal–Amur Mainline (BAM) railway by autumn 1984, a year ahead of schedule, have been jeopardized by a lack of understanding of the geology of permafrost. This year, the Soviet media have noted several instances where the rapid exploitation of geological research findings for the good of the economy, urged by successive five-year plans, has run into difficulties. Oil extraction from newly discovered fields in Siberia is far behind schedule, and it was revealed last month that the reservoir of the Cheboksary hydro-electric station on the Volga had caused an unexpected rise in the water-table between Cheboksary and Gor'kii, so that the water-table in Gor'kii has already risen to ground-floor level in some places.

Such problems seem endemic in a centrally planned system. Civil engineering works may be started without allowing sufficient time for preliminary surveys; and if one sector carries out a programme of "shock-work", others working at normal speed will appear to be lagging.

The trouble on BAM appears to be a result of the decision to bring forward the construction date, so that the last "golden link" could be laid to celebrate Revolution Day (7 November) this year. BAM, which has been hailed as one of the most important achievements of Soviet civil engineering, has been built in sections, and the running of trains on completed portions has already earned, its publicists claim, impressive sums from the Soviet economy. Looping far to the north of the (Tsarist-built) Trans-Siberian Railway, and thereby avoiding sensitive areas close to the Chinese border, BAM, when completed, is expected to offer fast and com-petitive freight rates for the container transport of goods from the Far East to Western Europe. The driving of the final link is therefore economically important.

The course of BAM was carefully planned — according to official reports — by a think-tank covering all the relevant scientific disciplines. The chief snag on the final choice of route was the Kodar range of mountains, through which a 2-km tunnel had to be drilled at an altitude of 3,000 m. When the completion date was brought forward, it became necessary to complete this tunnel by May 1984 (leaving six months for the track-layers to do their work), and the tunnellers forged ahead with such gusto that by 15 February, they had already excavated 1,600 m of tunnel.

Unfortunately, however, the concrete workers responsible for facing the newly dug surfaces made slower progress, and before they could catch up, lumps of rock began breaking away from the unfaced ceiling of the tunnel. It was eventually decided to reinforce the roof with metal structures, but this remedy was adopted too late to prevent a serious cave-in.

Serious recriminations have broken out as to who was responsible for the cave-in. The tunnellers blame everything on the "mysteries" of the permafrost. They have hinted, moreover, that the strata encountered were less hard than the geologists had supposed, and that although temperatures were monitored in the excavated tunnel, readings were taken only at floor level, and it was only later discovered that temperatures near the roof were considerably higher (around 12°C). The chief directorate for the BAM construction, however, tends to blame "sluggishness" in the concreting of the roof, while admitting that an elementary law of physics had been ignored — that at the temperatures that built up in the tunnel, permafrost gradually ceases to be permanent.

All the resources of Soviet society have now been mobilized: representatives of the Party bureau have been posted to "key sectors" and the concreting teams reinforced with Party cadres. The Ministry of Transport Construction has instructed the tunnellers to have the tunnel ready for traffic this autumn. The Chief Tunnel Construction Directorate, according to the Soviet newspaper *Sovetskaya Rossiya* considers this task "unrealistic". Officially, however, the schedule for driving the golden link remains unchanged.

Vera Rich

Optical microscopes

Reflector makes high price

A RARE reflecting microscope constructed in 1827 by John Cuthbert under the guidance of Dr Goring, the eminent Scottish microscopist, was sold last week in Sotheby's London sale-room for £8,000, about three times the price expected. The instrument was based on one produced several years earlier by the famous Italian experimenter Giovanni Amici, but instead of varying the magnification by a set of eyepieces, Cuthbert has provided a series of elliptical mirrors and Lieberkuhns. The mirrors are mounted in tubes to which the Lieberkuhns can be attached.

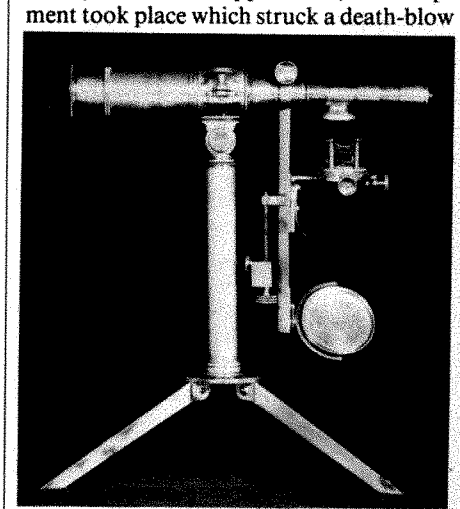
The reflecting microscope first made its appearance when the struggle for the achromatization of the microscope had almost been abandoned. Great strides had been made in the mechanical properties but there had been scarcely any improvement in the optical performance of the microscope since its invention two hundred years earlier.

Isaac Newton had certainly not helped matters by supporting the view that the construction of a colour-correcting achromatic lens system was not possible, and in 1672 he wrote to the secretary of the Royal Society outlining his ideas on how chromatic aberration could be overcome in a microscope by the use of a "reflecting piece of metal instead of an object glass".

Newton's preoccupation with a reflecting rather than with a lens system was influenced by his conclusion that refraction is always accompanied by dispersion, an unfortunate conclusion which went unquestioned for many years and which resulted in considerable delay before serious research was undertaken on the achromatic lens. As to Newton's microscope, if he ever had plans for its construction, they appear not to have materialized.

The first satisfactory reflecting microscopes made their appearance some 50 years later, and were by the Italian and London makers, Amici and Cuthbert. John Quekett, in his microscope treatise of 1848, points out the difficulties of making and using these instruments, but of the Cuthbert microscope, he says that it was (in its time) "the most perfect instrument manufactured in this country".

The success of Cuthbert's microscope was, however, short-lived. In 1830, just three years after its appearance, a development took place which struck a death-blow



The Cuthbert reflecting microscope

to all reflecting microscopes: the successful production of the long-awaited achromatic objective. For the first time, the conventional compound microscope began to emerge as an indispensable tool in science and industry. No longer was it merely a plaything for dilettante enthusiasts, who, to quote a nineteenth century historian, "regarded their costly instruments as precious toys rather than as tools for work and study".

Paradoxically the twentieth century has seen the re-emergence of reflecting systems for specialized use in ultraviolet or infrared regions of the spectrum; so even if Newton had been misled in his dispersion experiments, his pioneering work on the reflecting microscope was not altogether in vain.

Arthur Frank

Coronaries and diet

SIR — The article "You are not what you eat" (*Nature* **307**, 199; 1984) gives the seriously misleading impression that dietary modification cannot reduce the risk of coronary heart disease. In addition, the article contains many inaccuracies.

In the Coronary Primary Prevention Trial referred to in the article, all patients received a standardized diet which limited saturated fats and cholesterol and which was designed to lower blood cholesterol by 3–5 per cent. Both the treatment and the control groups achieved a 4 per cent plasma cholesterol reduction as a result. It is wrong to imply that the control group (no drug treatment) could not have done any better; controls showing a large dietary response were deliberately excluded. More restrictive diets have frequently been shown to reduce plasma cholesterol levels by 10–15 per cent.

You are incorrect in implying that only those in the study who reduced their plasma cholesterol to a level near the national average benefitted. Reduction of risk was directly and continuously related to the per cent reduction in cholesterol concentration. (You state that the group receiving the drug (treatment group) reduced plasma cholesterol by 17 per cent; the correct figure is 13.4 per cent. And you state that non-fatal myocardial infarctions were reduced by 17 per cent; the correct figure is 19 per cent.)

Although you did not distinguish between dietary cholesterol and dietary saturated fat, your article carries the clear implication that diet is unimportant in reducing the risk of coronary artery disease. The results of the trial clearly showed the benefit of lowering plasma cholesterol. In fact, it is almost standard practice for the patient to be treated with diet first, and a drug added only if diet fails to achieve a satisfactory reduction in plasma cholesterol. The results of the Oslo study, in which dietary modification and elimination of cigarette smoking achieved reduction in plasma cholesterol and a significant reduction in coronary artery disease, are consistent with the results of the Coronary Primary Prevention Trial and with our recommendation that patients with plasma cholesterol levels greater than 240 mg dl^{-1} should be carefully evaluated and should change their diet to reduce the level. Those whose plasma cholesterol remains elevated after a trial with diet are candidates for treatment with a drug. The suitability of this treatment must be an individual decision made by doctor and patient, based on accurate data on the patient's plasma lipoprotein levels. The trial indicates that vigorous treatment of this high-risk group will be effective if begun sufficiently early.

ANTONIO M. GOTTO

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Unfair on Illmensee

SIR — Your coverage of what you call "the Illmensee affair" continues to prove unfair. Or has it become common usage in *Nature* to report on the failure of anonymous "other researchers" who unsuccessfully try to "repeat Illmensee's experiments", as you do in *Nature* **308**, p.394? Knowing Illmensee for more than sixteen years and having worked as one of his fellow PhD students in the same room for more than a year, I am fully convinced of his personal and scientific integrity. If others fail to repeat his work successfully — what of it? Even Carl Lewis has not yet reached Bob Beamon's 8.90 metres.

ECKHARD LIEB

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Statisticians cleared

SIR — Following the recent article on the fallibility of the polygraph(*Nature* 29 March, p. 449) and the correspondence it generated (*Nature* 17 May, p.203), it is clearly now time to update that apophthegm which is the bane of a statisticians's existence: "There are lies, damned lies and lie detectors"!

D.O. CROWTHER

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Indian items

SIR — Your news report (*Nature* **307**, 307; 1984) rightly terms recent exhortations of the head of India's University Grants Commission (UGC) as utopian, although the UGC chief is to be credited for highlighting maladies responsible for academic backwardness in Indian universities. The bureaucracy and the politicians have strangulated university autonomy. There is interference in day-to-day running of the academic administration, as witnessed only a few months back when the vice-chancellor of a university resigned in protest at a state political authority encroaching upon his stand to maintain discipline in the campus.

In India education is a state matter. Except with regard to research, technical and scientific education, the education is entirely within the purview of the states. For this reason education has not developed a national perspective, with each state going its own way. The first step to improve matters would be to bring education under the category of the concurrent list of the Indian Constitution. This will enable UGC to intervene effectively to protect academic interests.

In the Indian university system, a vice-chancellor has a very important role to play in shaping the academic life of a university. But the recent trend is to appoint vice-chancellors mainly on political grounds. So vice-chancellors have failed to inspire the respect that students and teachers should normally have for a person who is supposed to be the leader of academic life of a university. In this context I may mention the report of Justice Mootham in which the choice of vice-chancellors was dealt with at length. Similarly, the Radhakrishnan commission on university education has not only described the quality of a person to be chosen as a vice-chancellor but also prescribed a procedure for appointment of vice-chancellors. Let academic merit be the guiding principle in the selection of a vice-chancellor. Furthermore, the highest authority in the government should be directly accessible to the vice-chancellor with no bureaucrat acting as a middleman.

Much of the erosion of university autonomy is done in the name of finances. This has reduced universities to just another section of the Education Department of the State Secretariat. There is no justification in placing a provincial civil servant designated as Financial Officer controlling the university finances. Such an officer has only a narrow bureaucratic outlook and cannot apprehend the need and priority of the academic challenges. Let the university be allocated "*x*" finances and details of its disbursement or priority in expenditure be decided by the academic body itself.

Recent approval accorded to a scheme of promotion of university teachers by UGC is incongruous to the recent pronouncements of the UGC chief. Only recently UGC has approved automatic promotion from reader to professor and from lecturer to reader on the basis of length of service (13 years as lecturer and 10 years as reader) of an incumbent. This move was rejected by the Indian Science Congress (*Nature* **307**, 100; 1984), but it is a worrying trend. Promotion should depend on open competition based strictly on merit without any bias tied to the length of service.

Finally, I am certain a great deal of practical wisdom is contained in the reports of various education commissions set up by the Government of India in recent years. The reports of the Radhakrishnan, Mudaliar, Mootham, Sen and Kothari commissions are gathering dust on various governmental shelves. The UGC chief could render an invaluable service by implementing relevant recommendations from each one of these reports, which can be implemented at the UGC level. Such actions would strengthen moves to persuade government machinery to take effective measures to save universities from academic deprivation. Genuine concerted efforts could turn a demoralized academic community into a professional force committed to seek and advance frontiers of knowledge and able to contribute its fair share in stimulating intellectual growth, nationally and internationally.

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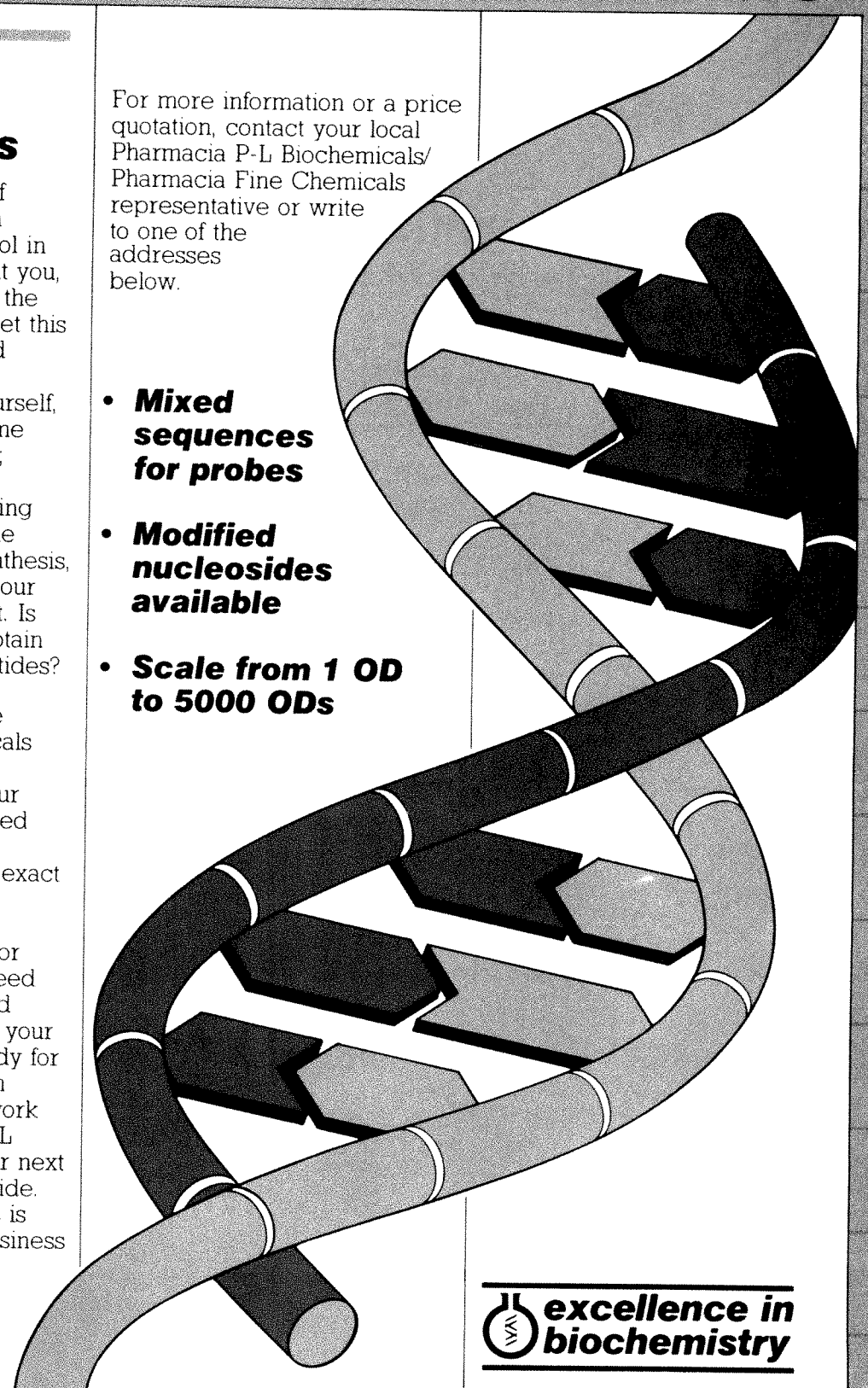
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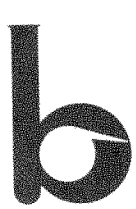
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Perils of too much disclosure

The New England Journal of Medicine *would have authors disclose commercial interests in their articles. The American Institute of Physics will tolerate prior disclosure. Why?*

WALL Street in the past year has been seriously damaged not by the unpredictability of the markets (from which it usually manages to profit) but in its reputation. A few months ago, it was alleged that an employee of the *Wall Street Journal*, a newspaper highly respected by the financial community, had been helping his friends to play the markets armed with the knowledge of what was about to appear in print.

Soon afterwards, an Arizona academic appeared on a television network, bad-mouthing G.D. Searle's new synthetic sweetener — and afterwards confessed that he had been selling Searle stock short in the expectation that its price would fall (which, surprisingly, it did not). Both tales are illustrations of what Wall Street and the other financial markets call "insider trading" — the exploitation of private knowledge by a few favoured people to make profits out of the ignorance of others. Their peculiar difficulty is that, in each case, the culprits are people not strictly subject to the regulations that govern the behaviour of recognized market makers.

For the financial markets and for governments, it has become a kind of nightmare to know how outsiders with inside knowledge should be prevented from cheating others. The agony seems now to have afflicted the *New England Journal of Medicine*, if anything even more highly respected than the *Wall Street Journal*. (At least, it does not fire off unsubstantiated charges that the Soviet Union is using genetic manipulation to manufacture biological weapons, as the *Wall Street Journal*, on the basis of a flimsy knowledge of what a gene is like, has recently been doing.) Last month, the *New England Journal*, in an article by its editor Dr Arnold J. Relman, raised the question whether the commercialization of medical research has not now become so "rampant" that those who publish scientific articles in the technical literature, but who also have interests in commercial companies, may suffer intolerable conflicts of interest (*New Engl. J. Med.* **310**, 1182; 1984).

The question is interesting and important. That biomedical research has become uncomfortably commercial is widely accepted, even by some of those who hold this to be unavoidable or even desirable, for the sake of the rapid development of a new technology. That the publication of scientific articles can sometimes affect the prices of particular stocks is also undisputed; the most obvious effects are those which follow the appearance of disappointing (negative) reports of trials with new drugs, but the phenomenon is not new. So how can journals ensure that they are not being misused by their contributors?

The *New England Journal's* suggestion, now opened for public discussion before being implemented as editorial policy, is that all contributors should declare direct commercial interests within what they seek to publish. An employee of, or one who has been helped financially by, an interested company should say so openly, but researchers who act as consultants for a company should make a declaration to the editor, who may afterwards ask that some of this information should be disclosed. On the face of things, this sounds sensible enough, entirely consistent with the contemporary fashion for publishing one's latest tax return before running for public office. But is the procedure necessary? And would it deal with the problem identified?

Especially since the latest surge of interest in biotechnology, most journals have found that full-fledged companies active in the field have been eager that their employees should clearly acknowledge who pays their salary. The declared motive is that the companies shall be better placed to recruit in the science labour market, but no doubt it helps on Wall Street that potential stockholders know the company is not asleep. The *New England Journal's* remedy would not change this state of affairs, but reinforce it. Practice suggests, however, that fast-moving corporations rely more on secrecy than on patent protection, with the result that the publication of important research is often delayed and, then, muffled.

In this climate, the most likely conflicts of interest are not those of contributors turned inside-traders but the following: members of the editorial staffs of journals may improperly use what they know about material not yet published, while contributors may tell lies in the interests of some secret sponsor. Fortunately, most journals are all too well aware of the dangers of even minor unauthorized disclosures. (*Nature* is still bruised by the angry withdrawal of an important article through the innocent transmittal of the report of a referee whose interpretation of the data was more interesting than the author's.) But the opportunities for financial gain are novel, and should be inhibited.

Contributors who may be tempted to shade the truth for hidden reasons are potentially a more serious problem. The *New England Journal's* recipe would help if only it could be enforced. The trouble is that those who may be the most tempted are the least likely voluntarily to disclose their interests. And the ultimate deterrent will be what it has always been — that those who shade the truth are eventually found out, and then damaged in reputation more seriously than their pockets can have benefited.

So, for the time being, *Nature* will not be following the policy with which the *New England Journal* is flirting, believing that present practices are most often sufficient to guard against abuse — and that the exceptions are those least easily dealt with by formal rules. Nor, for that matter, will *Nature* be following the new editorial policy announced by the American Physical Society (see *Physical Review Letters*, 11 June). On a quite different issue, that of the prior disclosure of the content of a scientific article, the society now says that it will not in future regard prior publication of the substance of an article in popular newspapers or magazines "with large circulations outside the physics community" as a bar to publication in its journals. It is shocking that this announcement should appear in the journal which, in the late Professor S.A. Goudsmit's time, most stoutly resisted the preference of many physicists for unrefereed publication on the front page of the *New York Times*.

The American Physical Society clearly does not appreciate what trouble this ill-thought policy will cause. Perhaps physicists are by now so dependent on the pernicious preprint system that they think their journals are archival from the start. But this new recipe is as neat a device as there could be for ensuring that important work is misrepresented to the public — one newspaper may sensationalize a hearsay account, the others will ignore the event for fear of seeming scooped. Even in the scientific press, broken embargoes may have the same disastrous consequences. This, in retrospect, is what may be made of the pallid account, in a recent issue of the journal *Science* (reference withheld), of a group of papers to be published in *Cell* and *Nature* in the next few weeks, and which may be the most important discovery so far this year. Thus will other tales of discovery be half-told if the American Institute of Physics is followed.

John Maddox

Physiology

Cardiac peptides and the control of sodium excretion

from G. A. Sagnella and G. A. MacGregor

PHYSIOLOGICAL experiments have repeatedly suggested the existence of natriuretic substances that increase the excretion of sodium from the kidney, probably by inhibiting the reabsorption of sodium along the renal tubule[1]. These factors are of particular interest partly because the amount of sodium in the body's extracellular fluid regulates its volume, and partly because of suggestive evidence of a link between the kidney's ability to excrete sodium and essential hypertension[1], a major risk factor for cardiovascular disease[2].

It has recently become clear that there are at least two distinct types of natriuretic substances. One, probably produced by the hypothalamic region of the brain, is an inhibitor of ($Na^+ + K^+$) ATPase, the enzyme which transports sodium across membranes, and might be a contributory factor to a rise in blood pressure[1]. The other, comprising peptides that have been isolated from the atria of the heart, has recently become the focus of attention for numerous laboratories throughout the world, although its physiological significance remains tantalizingly uncertain. The occasion for the sudden attention to the atrial peptides is their recent characterization and synthesis; moreover, as described on pages 717 to 726 of this issue of *Nature*, their precursor has now been identified and its sequence deduced.

Both neuronal and humoral mechanisms have been suggested[3] for the increase in urinary outflow that follows stimulation of atrial 'stretch' receptors[4]. Speculation that a novel endocrine system might be involved was stimulated by the observation that intravenous injection of rats with atrial, but not ventricular, extracts led to a striking natriuretic and diuretic response[5]. Moreover, the natriuretic activity is associated with endocrine-like secretory granules of the atrial cells[6,7], and the number of granules changes during water deprivation[8].

Early work suggested that the active material was a peptide[9-12] and, more recently, it was shown to be a mixture of peptides of different molecular sizes which are not only natriuretic but can also relax the smooth muscle of vascular tissue[13], causing vasodilation. A number of peptides — variously named atriopeptins, auriculins, cardionatrins and atrial natriuretic factors — have recently been purified and sequenced[14-20]; their structures are summarized in Fig.1. There are two striking conclusions. First, all the rat peptides seem to be derived from a common precursor, as suggested by earlier indirect evidence[9-12] and consistent with the isolation of a 73 amino acid peptide containing the atrial natriuretic sequence in its carboxy-terminal region[21]. Second, the one human peptide so far sequenced differs from the rat peptide by only one amino acid. All the peptides require an intact disulphide bridge for biological activity.

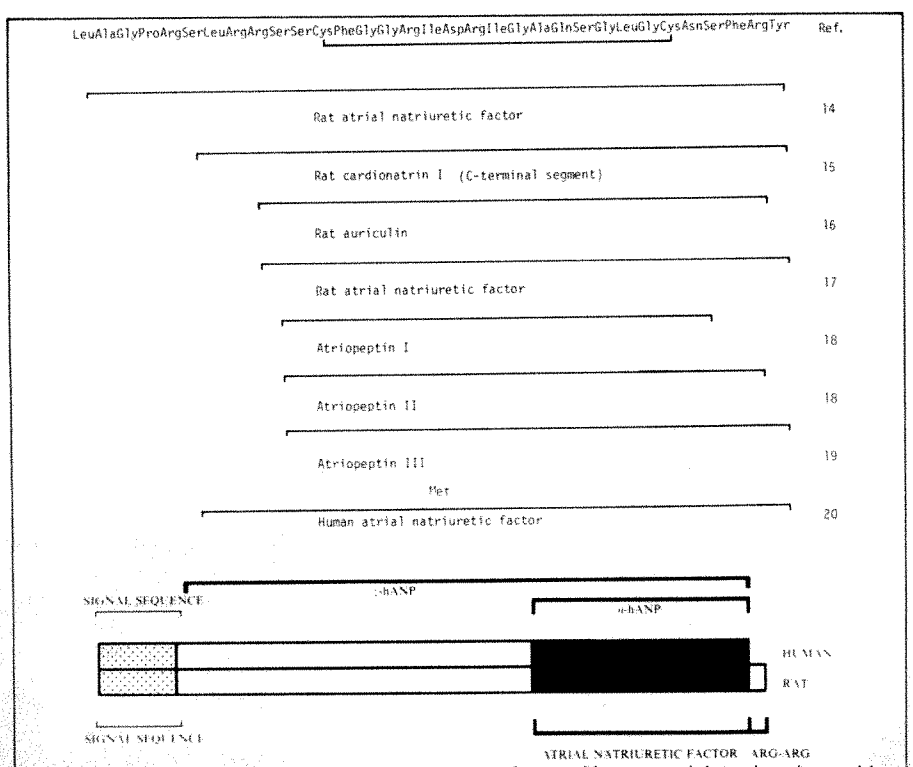


Fig.1 Amino acid sequences and proposed names of rat and human atrial natriuretic peptides (above) and organization of their precursors (below).

The suggestion of a common precursor for the peptides is now borne out by the sequences of precursor cDNAs of both rat and human atrial peptides reported by three groups in this issue of *Nature*. Both Yamanaka *et al.*[22] and Maki *et al.*[23] have cloned cDNA of the atrial natriuretic factor derived from rat atrial mRNA, and have determined its nucleotide sequence and deduced an amino acid sequence. The precursor is 152 amino acids long and contains in its carboxy-terminal region the amino acid sequences of all the atrial peptides reported to date (Fig.1). It also contains a likely signal sequence with a high content of hydrophobic amino acids in the N-terminal region, which is a characteristic feature of precursors of secretory proteins[24]. A remarkably similar sequence is reported by Oikawa *et al.*[25] for the cDNA encoding a precursor of the human atrial natriuretic peptide. (The deduced sequence contains not only the human atrial natriuretic peptide α-hANP, but also γ-hANP, a 126 amino acid peptide with the α-hANP sequence at its carboxy-terminus and which Oikawa *et al.* have identified in human atrial tissue — see Fig.1). Like the rat precursor, the human precursor contains a putative signal peptide sequence at its amino-terminus. There are some differences however — in particular, the Arg-Arg dipeptide following the atrial peptide sequence in the rat precursor is lacking in the human molecule. This may reflect differences in post-translational activation mechanisms.

The physiological function and importance of the atrial peptides have yet to be defined. Clearly, the fact that they have potent natriuretic and diuretic actions on the kidney as well as a vasodilatory effect raises the possibility of a role in the regulation of body fluids and blood pressure. Moreover, the peptides antagonize the vasopressor actions of the hormones noradrenaline[17] and angiotensin II[16]. Although limited, the available evidence is compatible with a hypothetical scheme in which an increase in extracellular fluid volume may serve as a trigger for release of atrial peptides into the general circulation (Fig.2); obviously, a number of questions remain to be answered.

How these peptides relax vascular smooth muscle and increase sodium excretion is not yet clear. They do not seem to act by inhibiting the ($Na^+ + K^+$)ATPase[12], and there is conflicting evidence as to their effect on the glomerular filtration rate — atrial extracts have no marked effect on it[26], whereas synthetic atrial natriuretic factor increases it slightly[16]. Atlas *et al.* suggest that the natriuretic effect results from the vasodilatory action of the peptide and a shift of blood flow to the medullary region of the kidney[16].

The characterization of the peptides at

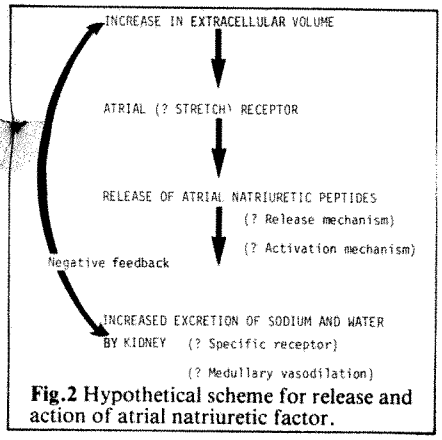


Fig.2 Hypothetical scheme for release and action of atrial natriuretic factor.

the molecular level and their subsequent synthesis provides a basis to investigate their mechanisms of action at the physiological and biochemical levels. There will undoubtedly be rapid progress in establishing radioimmunoassays to detect the presence of the peptides in the circulation and, if they are present, to test whether the circulating levels change with alteration of extracellular volume.

Clearly the potential roles of the peptides in disorders of electrolyte balance and in blood-pressure regulation remain to be clarified. For example, might Bartter's syndrome reflect excess atrial natriuretic factor and might hyporeninaemic-hypoaldosteronism be associated with a lack of it?

Finally, the relation of the atrial natriuretic peptides to other postulated, but as yet uncharacterized, natriuretic agents remains to be clarified. Teleologically, the existence of two natriuretic substances, one with vasopressor and the other with vasorelaxant properties, could allow the integrated control of both electrolyte and blood pressure homeostasis. □

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Optical physics

Generation of the shortest ever optical pulses

from Peter Knight

How many optical cycles do you need in a pulse for it still to be called a light wave? Ippen, Fujimoto and Weiner at MIT have just reported the generation and measurement of 16 femtosecond (1.6×10^{-14}s) optical pulses of centre wavelength 620 nm which contain just eight optical cycles (*Appl. Phys. Lett.* **44**, 832; 1984). They also report that they can see the colour of the light change as their high-repetition-rate laser apparatus compresses the optical pulses. These are the shortest light pulses ever generated and, since a molecule takes about 10 fs to vibrate, they open a new time-window in the study of ultrafast processes.

The generation of picosecond optical pulses has become fairly straightforward with the use of laser mode-locking. Because laser action is encouraged over as wide a frequency band as possible, many laser modes will oscillate at once. Mode-locking gives each mode a common phase so that constructive and destructive interference among the different waves leads to a regular train of pulses. Each of these pulses has a temporal width inversely proportional to the number of modes participating and thus to the laser gain bandwidth. For this reason a dye laser which can be excited over a wide frequency range can generate extremely short pulses. For example, a colliding-pulse ring geometry, developed at Bell Labs and now used by Ippen *et al.*, can generate reliable pulses of less than 100 fs duration.

The study of ultrafast phenomena requires even these short pulses to be further compressed in time. To achieve that degree of compression, Ippen *et al.* at MIT (and other groups in Bell Labs, IBM and Rochester) have stretched out the pulse frequency spectrum even more by 'chirping' the light so that the carrier frequency is swept. When this is followed by a dispersive element, the blue-shifted training edge catches up with the red-shifted leading edge and compresses the pulse.

Chirping can be produced by the nonlinear propagation of intense laser light in a liquid such as carbon disulphide. The intensity-dependent alterations in the refractive index of the liquid cause self-phase-modulation of the light. This method, proposed by Giordmaine at Bell Labs, has proved troublesome. The optical pulses can be chirped, but not in a reproducible or stable manner.

Nonlinear opticians have turned to single-mode glass fibres to produce stable uniform chirping. Light from a mode-locked laser can be focused into the small-diameter (about 10^{-3} cm) core of the fibre to give internal intensities large enough for nonlinear effects to be important. The nonlinear refractive index produces a phase-shift which depends on the instantaneous intensity. The variable phase-shift increases until the peak of the pulse arrives and then it diminishes. The instantaneous frequency shift is given by the time-derivative of the phase shift, so that frequencies in the leading part of the pulse are lowered while those in the training edge are raised. The size of this frequency chirp increases directly with the length of the fibre. This chirping, combined with dispersion in the fibre, is the basis of the 'soliton laser' developed by Mollenauer and Stolen at Bell Labs (*Opt. Lett.* **9**, 13; 1984). The spectrally stretched pulse, on emerging from the glass fibre, can be compressed in time using a dispersive grating pair technique which allows the blue-shifted trailing edge of the optical pulse to catch up with the red-shifted leading edge.

Ippen's new experiment uses a colliding-pulse, mode-locked, dye laser to produce a train of pulses each of 65 fs duration. Single pulses are selected and amplified to initial energies of 5 μJ with a repetition rate of 10 Hz, and then spatially filtered, collimated and focused into an 8 mm long polarization-preserving fibre, which broadens the spectrum of pulse by a factor of about four by nonlinear chirping. This is followed by a grating pair to compress the pulse to its final length of eight optical cycles. The pulses are detected by a second-harmonic auto-correlator which determines temporal length by measuring the spatial extent of the pulse of light.

In a femtosecond, light travels one-third of a micrometre, so femtosecond pulses can be used to study a whole range of ultrafast processes which, until now, have been unresolvable. In condensed matter physics, such pulses can be used to study transport processes in semiconductors, non-radiative relaxation, and molecular vibration and re-orientation. In photobiology and photochemistry, direct observations should be possible of energy dissipation, transport and dephasing. The generation of femtosecond pulses has stretched nonlinear optics to the limit. With only eight cycles left in a pulse of visible light, any improvement would seem to demand a shift to the ultraviolet. Meanwhile, the new results of Ippen *et al.* will be eagerly applied to a wide range of transient phenomena. □

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Microbiology

Proteins that bind the β-lactam antibiotics

from A.F.W. Coulson

A MEETING that is concerned more with the enzymes that form the targets for the β-lactam antibiotics than with the β-lactamases that destroy them, would not sensibly be called a 'β-lactamase workshop'. But the name was coined in 1975 for the first of the workshops; by the third*, held recently, the focus had changed.

The target enzymes can be identified in whole cells by their binding to ^{14}C-penicillin G. In *Escherichia coli* there are two classes of penicillin-binding protein (PBP). The low-molecular-weight proteins, PBP-4, -5 and -6, are not essential to the organism, are DD-carboxypeptidases and are believed to regulate the degree of cross-linking of the cell wall. The high-molecular-weight PBP-1a, -1b, -2 and -3 have already been shown to be essential to *E. coli*; real progress in understanding the behaviour of these membrane-bound proteins is now becoming possible through the cloning and sequencing of their genes.

The sequence of the PBP-3 gene, representing a protein of 588 amino acids, was reported a year ago by Y. Hirota (National Institute of Genetics, Mishima). PBP-3 displays both transpeptidase and transglycosylase activities, and the latter is not inhibited by penicillins. B. Spratt (University of Sussex) presented evidence that the transpeptidase activity is associated with the carboxy-terminal half of the molecule, whereas the amino-terminal half contains the transglycosylase activity.

J. Broome-Smith (University of Sussex) has sequenced the genes for PBP-1A, PBP-1B and PBP-5. 1A and 1B are highly homologous; otherwise the only extensive sequence similarities in this group occur in the region of the tetrapeptide -Ser-X1-X2-Lys-. Tetrapeptides of the same form also occur in low-molecular-weight DD-carboxypeptidases and in several examples of each of two types of β-lactamase. For β-lactamase it has been very thoroughly established that the serine residue is part of the active site, and is acylated by a β-lactam in the course of its hydrolysis. Other similarities in the sequences of these three groups of enzymes are also concentrated in the neighbourhood of the tetrapeptide sequence. It is clearly very tempting to suppose both that the serine residue is part of an active site with a similar role in the mechanisms of all the enzymes, and that all the genes are homologous, at least in parts.

A highlight of the meeting was the presentation of the first high-resolution X-ray crystal structure of one of the proteins that interact with penicillins: the 2.8Å structure of a *Streptomyces* DD-carboxypeptidase (J. Kelly, University of Connecticut). The enzyme contains two regions of secondary structure — a six-strand β-sheet and a group of five stacked α-helices. To add to the progress that has been made in the last four years in extending the sequence-based classification of β-lactamases, R. Ambler (University of Edinburgh) reported a partial sequence of the Zn-containing β-II enzyme from *Bacillus cereus*, still the only member of class B. And J.O. Lampen (Rutgers University) presented a partial sequence of its gene.

The most interesting mechanistic study was that described by R.F. Pratt (Weslyan University, Connecticut) who showed that acyclic esters of the form $Ar.CH_2.CO.NH.CH_2.CO_2R$ are hydrolysed by β-lactamases — the first non-β-lactam substrates to be discovered. Transpeptidation can occur from these substrates to suitable amines, which implies that acyclic amides must also be substrates (J.R. Knowles, Harvard University).

The induction of penicillinase in bacilli has unusual features which have made it a long-standing problem, a solution to which may be at hand now that clones of the entire penicillinase operons are available (Lampen). It seems clear that the main oddities in the induction arise because the inducer modifies the cell wall giving rise to a 'second messenger' within the cell; the genetic control itself may be entirely conventional. The nature of the modification to the cell wall remains mysterious, particularly since it can be brought about by a variety of chemically very different agents.

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*β-Lactamase Workshop, Holy Island, Northumbria, 4–6 April.

Astronomy

Quadrantid meteors

from David W. Hughes

IT is none too easy to gather data on the Quadrantid meteoroid stream. January 3 or 4, depending on our phase in the leap-year cycle, is the only day of the year on which the Earth intersects the stream; the point on the celestial sphere from which the shooting stars appear to originate has a declination of + 48.5°, so Quadrantids are best seen from high latitudes in the Northern Hemisphere where winter makes visual and photographic conditions seldom satisfactory; and at least one year in four is hampered by moonlight. Moreover, the Quadrantid stream is very narrow, the time between half-maximum flux rates being about 12 hours, and if the maximum occurs when the radiant is low in the sky, the proportion of the flux that can be observed by any method is much reduced. This factor limits optimum observations of the shower to one year in every four.

Radar methods can overcome two of the problems, permitting the observation of meteors in daylight and through clouds. They, however, suffer from their own kind of 'bad weather', because radio propagation conditions in the ionosphere can vary considerably as a function of solar cycle. Another problem with radar is the identification of the shower meteors. This is usually accomplished simply by subtracting an assumed background rate from the total observed rate.

One goal of meteor stream investigations is to unravel the disintegration process of the parent comet and the subsequent evolution of the meteoroid stream. A knowledge of the particle number density across the stream and around the stream orbit, and the way both of these vary as a function of particle size, would be very useful in that context. Hence the value of the determination by B.A. McIntosh, of the Herzberg Institute of Astrophysics, Ottawa, Canada, and M. Šimek, of the Astronomical Institute, Ondřejov, Czechoslovakia, of the long-term average cross-section of the Quadrantid meteoroid stream by analysing radar data obtained over nearly a quarter of a century (*Bull. astr. Inst. Czechoslovakia 35*, 14; 1984).

The Ondřejov and Ottawa radars are both pulsed systems and are similar in operation. Typical parameters are pulse length 10 μs, peak power 25 kW, repetition frequency 500 Hz. The antenna is fixed at an elevation of 45° and meteors within a line-of-sight distance of 400 km are recorded. Meteors are sorted into three duration classes, equivalent to median magnitudes of + 6, + 1.6 and − 1.0.

The practice of adding one year's data to the next is common with Quadrantid researchers but McIntosh and Simek warn that it is not without problems. These become obvious when the combined data for the time interval 1958–81 are compared with the well-observed year of 1979. There are three basic numerical values which represent any flux curve: the time of maximum flux, the rate of rise and the rate of decay. The last two can be combined to give a stream width which is usually defined as the change of solar longitude that occurs during the time when the stream is more active than half its maximum activity. The

authors noticed that the stream width from the combined data was twice that of 1979. The cause, they say, is the variation in the position of maximum, which can change by as much as 0.5° in solar longitude from year to year. So the annulus of Quadrantid meteoroids in the Solar System is not perfectly elliptical and deviations from this ellipse, at the descending node, can be as much as 7×10^5 km.

Contrary to the findings of many researchers, McIntosh and Šimek found that the maximum flux of bright meteors did not inevitably occur after the maximum flux of faint ones; in certain years the bright meteors preceded the faint ones. For all three meteor magnitudes the rate of rise of the flux to the maximum was slower than the subsequent fall. It was found, however, that the asymmetry increased as one considered brighter meteors. The theory of meteor stream production predicts that width should decrease as a function of meteor brightness. The authors found that the + 6.0 and − 1.0 magnitude meteors obeyed this rule but that the + 1.6 magnitude meteors had a wider flux curve than either of the others.

In conclusion, it is clear that the Quadrantid shower, currently both one of the most active showers and the narrowest, has many peculiarities. These can, in the main, be blamed on Jupiter. The ascending node of the Quadrantid orbit occurs very close to Jupiter's orbit. Also the mean period of the Quadrantids is approximately half Jupiter's orbital period. This causes the nodes of the Quadrantid orbit to regress by about 0.4° per century, leading to mass sorting in the shower (smaller meteoroids usually peaking earlier than larger ones) and introducing short-term resonance fluctuations which again have a size-dependent effect.

Jovian perturbation is very selective. The small sector of the stream that is in the vicinity of Jupiter every 11.86 years will be shifted by larger amounts than the rest of the stream. The velocities of the perturbed meteoroids will be changed slightly and they will redistribute themselves around the orbit, eventually forming a low-density tail preceding the main body of the stream. McIntosh and Šimek conclude that the degree of asymmetry so produced is to some extent a measure of the age of the stream.

This leads us to one of the main unsolved questions concerning meteor streams. How old are they? We know that the Quadrantids are old enough for the meteoroids to have spread out fairly evenly around the orbit and for the parent comet to have completely disappeared. But the stream is still young enough to retain the record of slimness. If only we knew its age our ideas about stream evolution could be placed on a much firmer footing. □

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Molecular biology

DNA gyrations in reverse

from James C. Wang

In the well-known double-helix structure of DNA, the two strands revolve around each other. The topological problem of separating the parental strands during replication, particularly when the double-stranded DNA is in the form of a ring and thus the complementary strands are two intertwined single-stranded rings, was recognized more than two decades ago[1,2]. The enzymes capable of solving such topological problems, DNA topoisomerases, are ubiquitous. They catalyse the transient breakage of the DNA backbone and the passage of DNA strands through the transient breaks[3-5] in two mechanistically distinct ways. The type I enzymes break and rejoin one strand at a time and catalyse the crossing of one strand through another; the type II enzymes break and rejoin a pair of strands in concert and catalyse the crossing of a double-stranded DNA segment through a double-stranded break. It is not surprising that both types of activities are found in an archaebacterium *Sulfolobus*, as reported by Kikuchi and Asai on page 677 of this issue of *Nature*; what is amazing, however, is that *Sulfolobus* also contains an entirely new subclass of type II topoisomerase[6].

Until now, two subclasses of type II topoisomerases had been found in eubacteria and eukaryotes. Bacterial gyrases, which fall into one subclass, are characterized by their ability to reduce the 'linking number' of a double-stranded DNA ring. The term 'linking number' is a topological quantity defined as the number of times one strand revolves around the other when the duplex ring is laid in a plane. If a DNA ring laid in a plane assumes the B-helical form, 10.5 base pairs make a complete helical turn and the linking number is the total number of base pairs divided by 10.5. When a duplex DNA ring is incubated with gyrase and ATP *in vitro*, the linking number is reduced by as much as 10 per cent below that of the most stable B-DNA structure. Gyrase achieves this energetically unfavourable reduction in linking number, termed 'negative supercoiling', by coupling DNA supercoiling to ATP hydrolysis.

The other subclass of type II topoisomerase includes those found in eukaryotes and phage T4 topoisomerase. These enzymes, though ATP-dependent, can only break and rejoin DNA to allow the passage of strands in such a way that the linking number of a duplex DNA ring 'relaxes' towards that of the most stable DNA structure, whatever its initial linking number; in this process, the chemical energy of ATP hydrolysis is not harvested by the DNA.

By contrast, the type II topoisomerase found by Kikuchi and Asai in *Sulfolobus* increases the linking number above that of the most stable DNA structure by coupling ATP hydrolysis to the positive supercoiling of DNA. 'Reverse gyrase', as Kikuchi and Asai call their enzyme, thus becomes the founder member of a third subclass of type II topoisomerase, and an unexpected one, because no positively supercoiled DNA has ever been isolated from cells in physiological conditions.

Since all three subclasses are ATP-dependent and catalyse the passage of one double-stranded DNA segment through another, their differences lie in the directionality of duplex cross-over. Strand passage catalysed by the gyrase and reverse gyrase is directional, though opposite in sign, whereas strand passage catalysed by the other subclass lacks directionality and the DNA relaxes into a thermodynamic valley.

The discovery of reverse gyrase raises a number of questions. First, is it a distinct enzyme rather than an altered form of a gyrase of the same organism that has somehow changed its direction of strand passage? Mechanistically, it is an intriguing question whether directionality can be altered by changing the quaternary structure of an enzyme or by modifying its subunits. The scanty information that is available comes from studies with *Escherichia coli* gyrase. In the absence of ATP, gyrase can relax negatively supercoiled but not positively supercoiled DNA. However, a complex containing the A subunit of gyrase and what is probably a proteolytic fragment of the B subunit of gyrase, can relax both negatively and positively supercoiled DNA[7,8]. Moreover, gyrase is stimulated to relax positively supercoiled DNA by the binding of the β,γ-imido-analogue of ATP[9]. Whereas such experiments show that it is possible to nullify the normal bias in the directionality of strand passage (relaxation of negatively supercoiled DNA) by the modification of a subunit of gyrase, it is more difficult to envision how strand passage could be switched from one direction to another by subunit modifications. The total lack of directionality in strand passage catalysed by phage T4 and eukaryotic type II topoisomerases is as intriguing as the directional strand passage catalysed by the other type II enzymes. In the case of the eukaryotic type II topoisomerases, the purified enzymes are homodimers. It is plausible that complexes between such enzymes and DNA have a twofold symmetry axis and that the symmetry abolishes any bias in the directionality of strand passage. The interesting question, then, is whether the binding of additional protein factors, or the

anchorage of the enzyme to a surface, can destroy such symmetry and make strand passage directional.

Is reverse gyrase unique to archaebacteria? Even with hindsight, there has been no hint of such an enzyme in eukaryotes. Interestingly, however, positively supercoiled plasmid DNA has been isolated from *E. coli* following inhibition of its gyrase by novobiocin or oxolinic acid, although this can be attributed to the formation of a complex between DNA and drug-inactivated gyrase molecules[10] — relaxation of a DNA when it is coiled around inactivated gyrase molecules is expected to increase its linking number[11]. Furthermore, DNA gyrase is overproduced in cells treated with gyrase inhibitors[12], which might explain the slow time course for the formation of positively supercoiled *E. coli* plasmid DNA following the addition of gyrase inhibitors[10]. The observation that *E. coli* plasmid DNA isolated from temperature-sensitive gyrase mutants at non-permissive temperature is not positively supercoiled[10,13,14] also argues against the existence of a reverse gyrase activity in *E. coli*. Nevertheless, the possibility of a reverse gyrase in eubacteria or eukaryotes cannot be discounted.

Why does *Sulfolobus* need a reverse gyrase in addition to its other topoisomerases? This is essentially the same question as why there are three topoisomerases in *E. coli*[15]. Whether some particular aspects of DNA in archaebacteria require a reverse gyrase is unknown; even the state of DNA supercoiling in such organisms remains to be determined.

If there is one philosophical point to be gleaned from the coexistence of supercoiling and relaxing topoisomerases, it appears to be that nature not only solves the topological problems that come with intertwining of strands in the double-helix structure of DNA, but also seems to take full advantage of the topological properties of supercoiled DNA. At least in eubacteria, there is strong evidence that intracellular DNA is negatively supercoiled, that the degree of unwinding influences many vital cellular processes including replication, transcription and certain site-specific recombinations[3-5], and that multiple topoisomerases are, perhaps, needed to regulate the degree (see ref.12 and refs therein). For eukaryotes, the first question is whether the evidence of their lacking supercoiling activity belies what actually occurs *in vivo*. Indirect evidence suggesting a supercoiled state in regions or subpopulations of intracellular DNA in eukaryotes has been accumulating (see, for example, refs 16–18). With the recent identification of the gene encoding yeast type II topoisomerase[19,20] and efforts of a number of laboratories in the search for other genes encoding eukaryotic DNA topoisomerases, powerful genetic tools can begin to complement the available biochemical approaches to answer this question. □

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Metallurgy

'Heavy' electrons in metals

from Tony Leggett

THE electrons in a typical metal interact quite strongly with the static crystalline lattice, with the vibrations of this lattice (phonons) and with one another. Many properties of nonmagnetic metals seem to be rather well explained, however, by a simple model, based on Landau's model of a Fermi liquid, in which the effects of these interactions are summarized by saying that the electrons behave as if they had an effective mass, m^*, which is different from their true mass, m, and a residual mutual attraction or repulsion. For almost all metals known until recently, the effective mass ratio m^*/m lies in the range 0.1–10 and the inter-electron interactions are not particularly strong. Great interest therefore attended the discovery of a class of metals which appear to be described by a Fermi-liquid picture but which have effective mass ratios of the order of 200 and, probably, correspondingly strong effective interactions. The interest was much increased when it was found that some of the metals of this class become superconducting at low temperatures, and is certain to be further heightened by the recent discovery that at least one of them, U_2Zn_{17}, undergoes a magnetic phase transition (*Phys. Rev. Lett.* **52**, 1551; 1984).

The systems in question are all compounds of a rare-earth element (usually cerium) or an actinide element (usually uranium) with one or more other metals: the most striking are $CeAl_3$, $CeCuSi_2$, UBe_{13}, UPt_3 and U_2Zn_{17}. From an atomic point of view, what they have in common is that the Ce or U atoms supply f-electrons, which are fairly tightly bound to the atom and would therefore be expected, in the solid, either to be strictly localized or to form a very narrow band, while the lighter elements supply p- or d-electrons which should be much more loosely bound; it seems probable that the electron states near the Fermi surface, which are expected to dominate the experimental behaviour, include both types. Probably the most striking experimental property shared by all these compounds is their enormous, low-temperature, electronic specific heats (C_{el}): while C_{el} is to a first approximation proportional to T as in ordinary metals, the ratio is of the order of 1 J K^{-2} as against the usual value, ~ 1 mJ K^{-2}. If interpreted in the conventional Fermi-liquid picture, this yields effective masses of the order of 200 electron masses; correspondingly, the 'Fermi temperature' above which the electrons are no longer degenerate is of the order of 10 K, compared with ~ 10^4 K for conventional metals. Although this huge effective mass could conceivably be due simply to the extreme narrowness of the band formed by the atomic f-electrons (the p- and d-electrons probably have much smaller effective masses and so contribute little to the specific heat), it seems very probable that it also has large contributions from strong electron–electron interactions.

While many of the experimental properties of the various compounds at temperatures above about 10 K are qualitatively similar, their low-temperature behaviour is strikingly different. $CeAl_3$ appears to undergo no phase transition of any kind down to the lowest temperatures reached (~ 0.02 K); $CeCu_2Si_2$ has a superconducting transition which depends sensitively on such factors as preparation method and pressure; UBe_{13} and UPt_3 become superconducting at about 0.85 K and 0.54 K respectively; while, as the new paper shows, U_2Zn_{17} undergoes a phase transition at 9.7 K to what appears to be an antiferromagnetic state.

In trying to make sense of this bewildering variety of behaviour it is tempting to turn for guidance to the one extended laboratory system of fermions in which we know that inter-particle interactions are very important, namely liquid ^{3}He. Indeed, some experimental properties of the heavy-electron metals, particularly the specific heat at intermediate temperatures, show some striking similarities to those of ^{3}He. One important feature of the behaviour of ^{3}He that is often believed to be characteristic more generally of strongly interacting Fermi systems is the part played by long-lived spin fluctuations, which arise from the fact that the liquid is 'nearly ferromagnetic' (or 'nearly solid').

Interestingly, when liquid ^{3}He becomes superfluid (the analogue of the superconducting transition in metals) the Cooper pairs form not, as in ordinary superconductors, with total spin S and angular momentum L equal to zero, but with $L = S = 1$. As a result, ^{3}He becomes an 'anisotropic superfluid' and has many unique properties. If, therefore, spin fluctuations are equally important in some or all of the heavy-electron metals, then it is tempting to speculate that these systems might become the first known superconductors to form Cooper pairs like those in ^{3}He; that is, the first (genuinely) anisotropic superconductors.

In fact, some of the experimental properties of UPt_3 and UBe_{13} have already been interpreted as supporting this hypothesis; however, it may be some time before a really clear-cut demonstration one way or the other is available. In this situation, the discovery of an apparently antiferromagnetic phase transition in U_2Zn_{17} is particularly intriguing: it demonstrates clearly that magnetic interactions can be extremely important in heavy-electron metals, yet simultaneously indicates that in this case at least they are not, *prima facie*, of the kind that is likely to give rise to anisotropic superconductivity. Clearly we have a lot to learn about the subtle interplay of interactions in these fascinating systems. □

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100 years ago

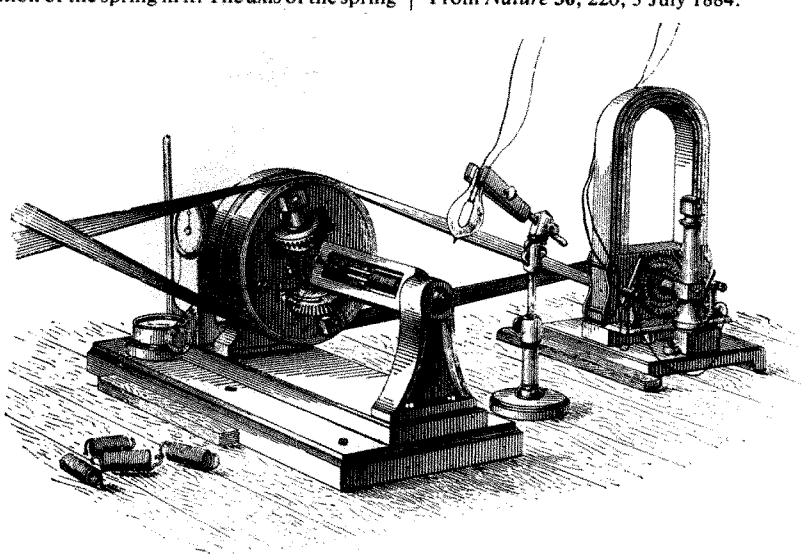
WORK-MEASURING MACHINES

UNDER this title a little *brochure* has recently appeared from the pen of the Rev. F. J. Smith, B.A., of Taunton, in which work-measuring dynamometers, or *ergometers*, of various forms are described. Amongst these machines there are many devised by the author himself, and some of these are of considerable interest and much originality.

The leading feature of this instrument is the position of the spring in it. The axis of the spring and of the shaft coincide; the result of this is that it is hardly at all affected by centrifugal force. When springs of slight pull are used and the ergometer is driven at a great velocity, the deformation is considerable, and would introduce considerable error in the result.

The deformation of the spring has been fully appreciated and therefore Rev. Smith has placed the spiral springs of his ergometer in cylindrical cases. When the spring is placed with its axis coincidental with that of the machine, no such error can be introduced and the friction of a spring against a case is avoided.

From *Nature* **30**, 220, 3 July 1884.



Smith's Transmission Ergometer

Mammalian development

Methods and success of nuclear transplantation in mammals

from Anne McLaren

NATURE's own nuclear transplantation device, the fertilizing spermatozoon, provides cogent evidence for the role of the nucleus in heredity. The demonstration that cytoplasm too has a part to play had to await the exploitation by Danielli and his colleagues of a microsurgical technique of nuclear transplantation in *Amoeba*[1]. Soon after, Briggs and King succeeded in transplanting nuclei from cells of frog larvae into enucleated frog eggs[2]. Their initial results suggested that nuclei become restricted in potency as development proceeds; but in a long series of experiments Gurdon and his colleagues showed that cell differentiation does not necessarily result in irreversible nuclear changes[3]. Thus nuclei from differentiated intestinal cells of tadpoles injected into enucleated frog eggs can be reprogrammed by the egg cytoplasm so as to support the development of adult frogs[4]; however, while nuclei from cells of adult frogs can support the development of tadpoles, the tadpoles fail to metamorphose into adults[5,6]. The widespread belief that clones of adult frogs have been produced by transplanting nuclei from adult tissue is thus fallacious.

Still more fallacious, of course, is the account of human cloning in Rorvik's *In His Image*[7]. Indeed, nuclear transplantation in mammals has proved very difficult to achieve. Since a mammalian egg is a thousandth or less of the volume of a frog egg, this is hardly surprising. Microsurgical techniques have been devised both to remove single pronuclei from mouse eggs[8-10], giving haploid embryos, and to transplant embryonic nuclei into fertilized mouse eggs, giving a tetraploid chromosome complement[11]. Both procedures are followed by cleavage to the blastocyst stage, but attempts to combine the two procedures by transplanting nuclei into mouse eggs from which both pronuclei have previously been removed invariably lead to disintegration of the eggs (J.A. Modlinski, personal communication). Attempts have also been made to introduce nuclei from eight-cell embryos into unfertilized rabbit eggs[12], either by microsurgery or by virus-mediated cell fusion, but the success rate is low, and no more than two to three cleavage divisions are ever observed. Virus-mediated fusion of somatic cells with mouse eggs has shown equally little promise[13,14].

The first report of mice born from nuclear transplantation came from Illmensee and Hoppe[15]. The trauma of two operations on the same egg was avoided by injecting donor nuclei into fertilized eggs and then immediately sucking out the two pronuclei of the recipient, using the same pipette. The donor nuclei were taken from blastocysts: if from inner cell mass cells, the recipient eggs sometimes developed to term but if from trophectoderm cells development did not proceed beyond mid-cleavage. A similar difference was reported for nuclei transplanted to non-enucleated fertilized eggs to produce tetraploid embryos[16]: eggs receiving inner cell mass nuclei developed into blastocysts, but those receiving trophectoderm nuclei blocked at the eight-cell stage.

The microsurgical technique of Illmensee and Hoppe has not yet been successfully carried out in any other laboratory, though it has been attempted by at least four groups, and probably more. In contrast, a technique devised by McGrath and Solter[17] has already been used with success in our own laboratory[18] and another[19]. Their technique avoids the necessity of penetrating the plasma membrane of either the donor or the recipient egg by sucking out the pronuclei surrounded by a piece of plasma mem-

brane — a karyoplast. The donor karyoplast, together with some Sendai virus to promote fusion, is injected under the zona pellucida of the enucleated recipient egg, with which it fuses. The yield of live mice is very high when fertilized eggs are used as donors, but no successful development beyond the blastocyst stage has so far been reported with donor nuclei from older embryos.

One application of the technique has been to exchange pronuclei between fertilized eggs of different strains, so as to investigate whether the basis for maternal effects transmitted via the egg resides in the nucleus or in the cytoplasm at the time of fertilization. In this way expression of the stage-specific embryonic antigen SSEA-3 has been shown to depend on the egg cytoplasm[20], while the lethal effect of the hairpin tail mutation when transmitted through the mother seems to be a property of the pronuclei[21].

Another application has been to exchange pronuclei between fertilized and parthenogenetically activated mouse eggs in an attempt to understand the mysterious failure of parthenogenotes, whether haploid or diploid, to develop to term. When pronuclei from eggs parthenogenetically activated by alcohol treatment were transplanted by the McGrath and Solter technique into enucleated fertilized eggs, the embryos did not survive to term; but pronuclei from fertilized eggs, transplanted into parthenogenetically activated and then enucleated eggs, produced live mice[18]. This result suggests that the defect lies in the pronuclei, not in the cytoplasm. In contrast, when inner cell mass nuclei from parthenogenetic blastocysts were microsurgically transplanted into enucleated fertilized eggs, using as donors spontaneously activated LT/Sv embryos, Hoppe and Illmensee reported that they developed normally[22].

Diploid parthenogenetic embryos are a special case of embryos with two maternal and no paternal genomes: such embryos can also be derived by removal of the paternal pronucleus, followed by doubling of the remaining maternal pronucleus. Such homozygous diploids, whether formed from the maternal or paternal pronucleus, have been reported to develop to term[9], but attempts to confirm these results have not been successful[8,23,24].

Using the McGrath and Solter technique to exchange single pronuclei, it has recently been shown that normal development does not follow the addition of a second maternal pronucleus to a haploid parthenogenote, whereas the addition of a paternal pronucleus results in a viable embryo[19]. Also, while a paternal and a maternal pronucleus taken from fertilized eggs and recombined will support development, two paternal or two maternal pronuclei fail to do so[25]. Unless some extrachromosomal component of the pronucleus plays a hitherto unsuspected role in development, these two pieces of work suggest that normal development requires both a paternal and a maternal chromosome set.

Only when we understand the reasons for the developmental failure of certain categories of embryo will it be possible to assess the significance of these results as a whole. In attempting to resolve the apparently conflicting lines of evidence summarized above, certain points must be borne in mind. When pronuclei are sucked out with a fine pipette, some or all of the pronuclear membrane may remain behind even though the pronuclear contents are removed. When nuclei are transplanted, either by microinjection or cell fusion, some cytoplasm is also transferred. Eggs artificially activated by exposure to alcohol cannot necessarily be equated with spontaneously activated LT/Sv eggs. Finally, inner cell mass nuclei cannot be equated with pronuclei: it may be that by the blastocyst stage, developmental potential has become restricted; alternatively, some developmental block affecting the nuclei of parthenogenetic eggs may have been overcome by this stage.

A precedent is seen in the *o* maternal effect lethal of the axolotl. This involves a cytoplasmic effect such that eggs from homozygous females, that would otherwise die during gastrulation, can be rescued by injection of normal oocyte cytoplasm or nuclear sap at the one-cell stage[26]. Transplantation of normal nuclei into enucleated *o/o* eggs is followed by normal development if the donor nuclei are taken from mid-blastula or later embryos, but not if they are taken from early blastulae, suggesting that some heritable change takes place in the axolotl nucleus during the blastula stage[27].

What of the future? Now that mouse strains carrying mitochondrial variants[28] are available, the use of recipient eggs carrying a mitochondrial marker will provide unequivocal evidence that a mouse is indeed derived from a nuclear transplant, just as the one-nucleolus marker provided similar certainty for amphibian nuclear transplantation[3]. The McGrath and Solter technique can be used to analyse any maternal effect exerted via the egg[29], and also perhaps to examine the roles of nucleus and cytoplasm in oocyte maturation. But only if it can be extended to use nuclei from later stages of development, or if the Illmensee and Hoppe nuclear transplantation technique can be successfully repeated, will the type of analysis pioneered in Amphibia also become possible in mammals. Does the developmental potency of nuclei become irreversibly restricted in the course of differentiation? Can somatic nuclei be reprogrammed, perhaps by transfer through several successive oocyte hosts? Can even adult nuclei be persuaded to revert to their ancestral totipotent condition? If not, why not? Perhaps irreversible gene rearrangements during somatic development, of the type undergone by immunoglobulin genes in the development of antibody-producing B cells, are more common than we realize. Mammalian systems may throw light on these crucial questions, at present unanswered even in Amphibia.

The potential rewards in animal breeding are great, with the cloning of prize-winning animals as the ultimate goal. Unless it becomes possible to use adult nuclei for renucleation of enucleated eggs, it will not be possible to clone champions, as such. But if large numbers of genetically identical embryos could ever routinely be produced by breeders, using inner cell mass nuclei to renucleate eggs, most could be stored in frozen form while waiting to learn if a champion developed from one that was taken to term. If so, the frozen embryos could be thawed and transferred to foster mothers as required.

For human embryos, nuclear transplantation has a bad name because of the science fiction overtones of cloning; but for couples at risk of transmitting a genetic defect to their progeny, it could provide a method of screening that did not involve, as present methods do, termination of the affected pregnancies. If several eggs were recovered from a woman and renucleated with inner cell mass nuclei from one of her own embryos, a few could be frozen, while the genetically identical remainder were screened for a genetic defect, by chromosome analysis, DNA analysis or other biochemical methods. In this way it would be possible to assure the woman that any embryo transferred to her uterus from the frozen stock would be free of the screened-for genetic defect. □

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Direct land/sea correlations in the last interglacial complex

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Isotopic and pollen analyses of an eastern Atlantic core permit the first direct correlation of substages 5e and 5c of the marine climatostratigraphical system with the Eemian and St Germain I, respectively, of the classical Grande Pile pollen sequence (north-east France). The vegetational sequences during the warm intervals of the last interglacial complex (125,000–75,000 yr BP) indicate that climatic deterioration occurred late on the mid-European continent after the initiation of glaciation over continental masses in high latitudes.

IN the reconstruction of the global climatic patterns of the succession of glacial and interglacial periods of the Quaternary, the correlation of deep-sea records and of continental data is of the first importance. Because the $\delta^{18}O$ signal in continuously deposited deep-sea records is chiefly determined by global ice-volume[1,2], it is globally synchronous in the open oceans and thus provides a basic climatic stratigraphy for oceanic events.

Unfortunately, land-based records cannot be easily correlated with the oceanic records. First, there are several independent climatostratigraphical systems[3] based on palynological data, studies of loess–soil succession and of the geomorphological and sedimentological features of terraces and moraines. Second, because many of the data extend beyond the ^{14}C time scale, their chronology is only poorly controlled. Finally, the discontinuity of continental records impedes inter-regional correlations. The result is that there is no reliable linkage between continental units and the now-standard oceanic ^{18}O succession[3,4].

This is true even for the last interglacial period (125,000–75,000 BP), which most closely resembles the present in climate and which might serve as a guide to future climatic conditions. Thus, in palaeoceanographic studies, oxygen isotopic data[5–8], changes in marine faunal assemblages[9–12], and carbonate curves[13], show that the last interglacial interval[5,7,9,14,15] (125,000–75,000 yr BP), which was the last globally warm period before the present, was characterized by a succession of three main warm phases separated by two cold phases. This palaeoclimatic history has been also recorded in ice cores[16,17] and in the succession of coral terraces[18,19].

In the oxygen isotope stratigraphy, the last interglacial interval corresponds to Emiliani's stage 5 (ref. 5) in which the oldest subdivision (Shackleton's substage 5e; ref. 7) can be regarded as the warmest period of the whole interglacial complex and at least as warm as today. Fragmentary continental records have revealed that a climate as warm as the present one certainly occurred during the Eemian stage in northwestern Europe. On the basis of similarities between climatic conditions recorded in deep-sea cores and continental sequences, Shackleton[7] first suggested that the northwestern European Eemian interglacial was the continental equivalent of substage 5e. But now it seems that in moraine systems there were three stratigraphically separate Eemian interglacials[3,4], and that numerous truncated Eemian deposits (including the type Eemian of the Netherlands) cannot be correlated with stage 5e. In fact, the best and most complete climatic succession through the last interglacial complex has been obtained later from the long continental sequences of Grande Pile (northeastern France)[20] and Tenagi Philipon (northern Greece)[21]. These sequences reveal that there was a succession of three warm intervals during the interglacial, as observed in the deep-sea record. Nevertheless, the striking resemblance between the climatic evolutions observed in both land and sea sequences does not necessarily imply precise synchronism between the successive parallel climatic events. Indeed, discrepancies remain concerning the real correlation of French Eemian, Greek Pangaion and subsequent warm periods with the oceanic stratigraphical framework of the last interglacial complex[20,22–25].

In view of the difficulties which are encountered in continental sequences, marine palynology seems to offer the most valuable method for direct land/sea correlations. It has recently been shown that pollen and spores contained in deep-sea sediments neighbouring coasts may indeed reflect the adjacent continental vegetation[26–28]. Moreover, marine palynological sequences without major lacunae can be directly correlated *in situ* with deep-sea isotope stratigraphy. Thus core records from the north-eastern Pacific[29] and Bay of Biscay[30] permit direct marine/continental correlation between events of the last deglaciation (20,000 yr BP–present). In the same manner, off the Washington–Oregon coast[31] and in the Arabian Sea[32], the correlation of the vegetational evolution of the last interglacial complex with the isotopic subdivisions of stage 5 has also been demonstrated.

By means of palynological studies from a deep-sea core located off Iberian coasts in the Atlantic ocean, I aim here:

(1) To determine, for the first time, the vegetational and palaeoclimatic evolution of the adjacent southwestern European continent during the last interglacial complex.

(2) To correlate continental events recorded in the present study, during isotopic substages 5e, 5d and 5c, with the lower part of the continuous palynological diagram from the Grande Pile peat-bog, which is today the nearest continental site to the deep-sea record studied.

(3) Thence, to correlate directly the lower part of Grande Pile sequence with the standard isotope $\delta^{18}O$ stratigraphy, which has been established in the marine core.

(4) To determine precisely the relation between the onset of ice growth phases in polar areas and the continental response in southwestern Europe to climatic changes, which occurred at the transition between warm isotopic substages 5e, 5c and the full glacial conditions of substages 5d and 5b.

Isotope and pollen records in core SU 8132

Core SU 8132 was recovered near the continental slope 100 km off the Iberian coast, near the Spanish–Portuguese border (42°06′N, 09°41′W, 2,280 m). As has already been shown in the Bay of Biscay[26], deep-sea sediments at the same distance from vegetated landscapes, contain numerous pollen and spores, the distribution of which reflects the regional vegetation of the adjacent continent.

Oxygen isotope records were made on the basis of analyses of the planktonic foraminifer *Globigerina bulloides* (>250 μm fraction)[2]. Because benthic $\delta^{18}O$ records are less influenced by

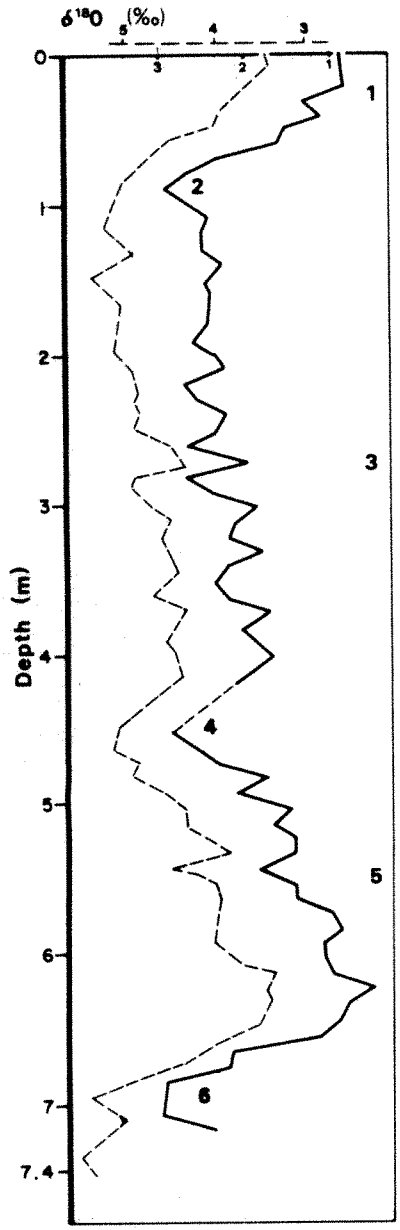


Fig. 1 Oxygen isotope, $\delta^{18}O$, record of planktonic foraminifer *Globigerina bulloides* (solid curve) and benthic foraminifers after normalization to *Uvigerina peregrina* (dotted curve) in core SU 8132, after J. C. Duplessy (personal communication).

local effects than planktonic ones[33], benthic foraminifera were also analysed to obtain a better isotope stratigraphical framework, primarily reflecting global ice volume evolution at high latitudes[34,35].

The $\delta^{18}O$ record (Fig. 1) shows that core SU 8132 represents a high sedimentation rate and gives a long continuous sequence with the classical development of Emiliani's stages[5] from 6 to 1. Unfortunately, during stage 5, Shackleton's upper substages[7] 5b and 5a are difficult to determine because of the low rate of sedimentation at that time, and/or the disturbed nature of the sediment. By contrast (Fig. 2), substage 5e is well developed from 680 to 580 cm, representing a high sedimentation rate for this period (100 mm kyr^{-1}). The glacial/interglacial change at the 6/5e boundary is marked by a major shift at 680 cm corresponding to Termination II of Broecker and Van Donk[14]. Substage 5c is also identifiable between the two major $\delta^{18}O$ increases at 540 cm (substage 5d) and 490 cm (lower boundary of 5b). There is another major $\delta^{18}O$ shift corresponding to the stage 4/stage 3 boundary at 420 cm.

In core SU 8132, palynological analysis has only been applied to the interval between stages 6 and 3. Samples were treated according to classical methods: HCl, HF and heavy liquor, and sieving on 15–20 μm mesh. Some 100–200 grains were counted (excluding *Pinus* (pine) pollen). To delete the overrepresentation of *Pinus* pollen grains that affects pollen profiles in marine sediments[36], percentage evaluations on Fig. 2 were based on the total pollen grains, including AP (arboreal pollen minus *Pinus* pollen), and NAP (non-arboreal pollen) and spores of ferns. The pollen diagram shows warm intervals in which temperate tree pollen dominates, alternating with cool intervals in which non-arboreal pollen is relatively more important.

At the end of stage 6, pollen spectra reflect an open cool vegetation (Graminae, Cyperaceae, Ericaceae, rare *Artemisia* and other Compositae) representing up to 95% of the basic sum. Arboreal pollen (except for *Pinus*) is very rare. At the boundary of stage 6/substage 5e, Termination II is very well marked by the immigration of *Betula* (birch) and the first appearance of *Quercus* (oak) (pollen zone 2). The early part of substage 5e is characterized by the maximum relative value of *Quercus* (pollen zone 3). Abundant *Quercus* is accompanied by a noticeable increase in *Carpinus* (hornbeam) (pollen zone 4), followed in the latter part of substage 5e by the well-marked development of *Abies* (fir) and a slight spread of *Betula* (pollen zone 5). At the 540-cm level, the pollen profile reflects the return of cold conditions with a major drop in the AP curve directly correlated with the cold event 5d (pollen zone 6). The sharp $\delta^{18}O$ decrease which followed (substage 5c) is characterized by the dominance of temperate tree pollen, with an early *Quercus–Carpinus* succession accompanied by a slight increase in *Abies* (pollen zones 7, 8 and 9).

Another short-lived disappearance of temperate forest occurs in pollen zone 10. Pollen zone 11 is characterized by a slight immigration of *Quercus*, but the landscape is dominated throughout by herbaceous plants. As has been previously noted, the sedimentary sequence of this part of core SU 8132 seems disturbed and, therefore, the vegetational record could be either discontinuous or mixed.

Pollen zones 12 and 13 at the top of the sequence show an alternation between assemblages without forest development, which corresponds to stage 4, and an increasing proportion of temperate trees (*Betula, Quercus, Abies*) correlated closely with the early part of stage 3.

Thus marine pollen analyses from core SU 8132, being directly correlated with oxygen isotope stratigraphy, show for the first time that during the early and middle part of stage 5, the palaeoclimatic evolution of the neighbouring western Iberia was characterized by a succession of two warm phases (5e = pollen zones 2–5; 5c = pollen zones 7–9) separated by an abrupt and very cold interval (5d = pollen zone 6).

Moreover, during the two older warm periods of stage 5 (5e and 5c) a logical succession is observed in the immigration of various temperate trees starting after the glacial episodes (6 and 5d)—early development of *Betula* soon replaced by the immigration of *Quercus*, then marked extension of *Carpinus* and a late increase in *Abies*. This vegetational evolution is in good agreement with the main criteria taken in continental pollen sequences to charcterize, from a palynological point of view, a complete interglacial cycle[25,37–40]. By comparison, the early part of stage 3, which is marked by the simultaneous immigration of *Betula, Quercus, Abies* and lack of *Carpinus*, must be interpreted as an interstadial period.

It can be seen that the pollen assemblages from substage 5e and the pollen assemblages from substage 5c are generally similar but 5e slightly differs from 5c in the more important role of *Abies* in the latter part of the episode.

Correlation with continental sequence

Many continental sequences covering the last interglacial climatic cycle in northwestern Europe have been studied by means of pollen analyses[41–46]. They indicate that a warming period, for example, the classical Eemian[47,48] in Holland, occurred

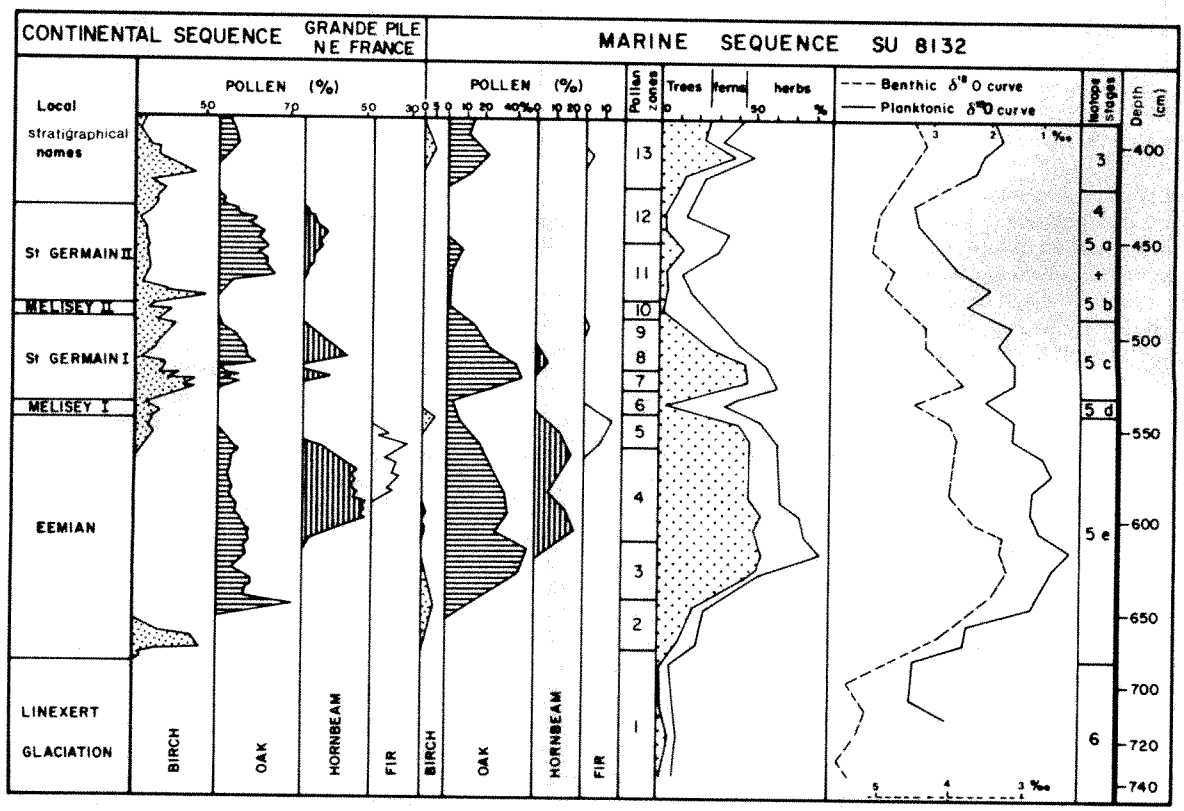


Fig. 2 Direct correlation of $\delta^{18}O$ stages with the continental sequence of Grande Pile by means of the marine palynological sequence of core SU 8132. Continental sequence after ref. 20. Benthic and planktonic $\delta^{18}O$ after J. C. Duplessy (personal communication). In the marine sequence, the relative frequency for pollen of tree species is expressed as a percentage of the total pollen+spores, excluding pine pollen.

below the last Weichselian glaciation. Unfortunately, few continental deposits apparently contain a continuous pollen record extending over the entire last interglacial complex. Lack of evidence for a complete record of subsequent climatic evolution up to the present interglacial, and absence of accurate absolute dating methods have often posed the problem of their real place in the interglacial/glacial succession of continental climato-stratigraphical systems[3,4,49]. One of the best and most complete sequences known at present is from the Grande Pile in north-east France[20]. Figure 2 shows that between the end of a long glacial period (? Saale, ? Warthe) and the beginning of the Würm glaciation, which is radiometrically dated at ~70,000 yr BP (ref. 50) there were three temperate intervals, namely Eemian, St Germain I and St Germain II, separated by two very cold phases, Melisey I and Melisey II (Fig. 2). Furthermore, the early warm period (Eemian) is characterized by the successive extension of the tree species *Betula*, *Quercus*, *Carpinus*, and *Abies*, very similar to that recognized in core SU 8132 during isotopic substage 5e. In the Grande Pile record St Germain I slightly differs from the Eemian mainly in the lack of *Abies*, in the same manner that the development of *Abies* in marine substage 5c is less important than in 5e. As in the marine record during 5d, palynological evidence from Grande Pile shows that Melisey I was an abrupt and very cold period which separated the two older warm intervals of the interglacial complex. However, some Eemian and St Germain I interglacial features do not occur in the deep-sea record: there is a lack of *Picea* (spruce) and *Corylus* (hazel) phases, and a lower species diversity of herbaceous taxa. These can be explained by taking into account the southerly position of core SU 8132, the selective effect of the regional (but not local) origin and marine transport processes of the marine pollen assemblage[26]. Therefore, despite some slight differences, the marine pollen diagram exhibits the same climatic trends, and a similar general succession of vegetation landscapes as in north-east France and there now seems to be no doubt that Woillard's Eemian and St Germain I interglacial periods correspond, in core SU 8132, to substages 5e and 5c respectively, and that Melisey I correlates closely with substage 5d.

Despite some controversies in earlier estimates[15,51–53], there is now general agreement on assigning dates of ~125,000 and 75,000 yr BP to Emiliani's stage 6/5 and 5/4 boundaries respectively[54–56]. Radiometric dating of coral terraces[14], and $\delta^{18}O$ time scales determined by assuming a constant sedimentation rate, are so similar that a correlation of the three $\delta^{18}O$ minima 5e, 5c, 5a and high sea level stands on Barbados at about 125,000, 104,000 and 80,000 yr can be demonstrated[15]. Therefore, extrapolated ages for the two cold peaks (5d, 5b) can be estimated as 110,000 and 90,000 yr BP, respectively.

These peaks are synchronous within the mixing time of the world's ocean waters, and the marine palynological evidence of SU 8132 allows us to conclude that Woillard's Eemian and St Germain I interglacials are correlated with Barbados I and Barbados II, while the lower boundaries of Melisey I and II can be placed at ~115,000 and 95,000 yr BP respectively.

This direct correlation between deep-sea chronology and the lower part of the continental stratigraphical sequence from Grande Pile confirms and gives precision to the previously hypothetical correlations suggested by Woillard and Mook[50] for this interglacial complex. Conversely, the results of the present study invalidate correlations of the Eemian interglacial with the whole of stage 5 or with substages 5e, 5b, 5c combined, as suggested by Morner[23,24].

Continental vegetation and ice sheet growth

In core SU 8132, the oxygen isotopic curve based on benthic foraminifera shows that after a well marked minimum in the lower part of substages 5e and 5c, $\delta^{18}O$ values began to increase within the middle part of these two warm interglacials long before the onset of glacial substages 5d and 5b (Fig. 2). This would indicate that an early phase of ice growth took place in polar latitudes before the end of both Eemian and St Germain I time. From numerous North Atlantic cores, Ruddiman *et al.*[57]

observed a similar trend across both 5e/5d and 5a/4 transitions.

Moreover, pollen data from core SU 8132 (Fig. 2) clearly indicate that the beginning of each ice growth (at 610 and 520 cm) is directly correlated with pollen zones 4 and 8 respectively, which are both characterized by the spreading of *Carpinus*. From pollen assemblages recorded in continental deposits during past interglacials it appears that the classic *Carpinus* phase is often marked by the presence of thermophilous plants such as *Ilex*, *Buxus*, *Hedera* and *Viscum*[20,40,41,47,58]. Thus, it may be assumed that the *Quercus–Carpinus* vegetational succession does not indicate climatic deterioration but, on the contrary, general stability of warm temperatures during the climatic optimum of each interglacial period. According to Andersen[58] this classical succession of vegetation landscapes could be conditioned by soil evolution rather than by climatic change. In fact, from the SU 8132 sequence it appears that temperatures in southwestern Europe probably began to decrease only with the immigration of *Abies*, which occurred at the end of both interglacial intervals, but that full glacial conditions, marked by high amounts of herbaceous plants, did not start before major ice volumes had been accumulated in Arctic zones during substages 5d and 5b. All these data indicate that the initiation of Boreal ice caps, during the second half of the Eemian and St Germain I interglacials, did not correlate with a temperature decrease in southwestern Europe, probably because an ice cover was not yet established over Scandinavia.

This pattern agrees well with the evolution of palaeotemperatures in the northeastern Atlantic ocean at interglacial to glacial transitions during stage 5. By means of matched isotopic and marine faunal data, Ruddiman and McIntyre[59] pointed out the existence between 40° N and 60° N of warm, eastern North Atlantic sea-surface temperatures during the first half of rapid ice growth. They speculated that along with low summer insolation, which occurred at the 5e/5d and 5a/4 transitions, an 'interglacial' warm Atlantic Ocean might be a necessary condition for delivering moisture to high latitude areas, thereby leading to the initiation of extensive glaciation.

Moreover, according to Royer *et al.*[60], the cooling linked to the insolation change, which occurred around 115,000 yr BP, was not uniformly distributed over the northern high latitudes, but has a well defined maximum, combined with increased precipitation, over North America. These data suggest that initial high-latitude glaciation was established on the western side of the North Atlantic Ocean. In this context, evidence from the SU 8132 record indicates that the initiation of the Laurentide ice sheet did not cause a temperature decrease in southwestern Europe, and probably excluded the simultaneous inception of ice caps in Scandinavia. It is only later, at the end of the interglacial periods, that the disappearance of *Carpinus* forest and extension of *Abies* phases probably indicates the mid-European continental response to the onset of glaciation over Scandinavia.

Finally, the present study provides additional data on a parallel climatic evolution between the eastern North Atlantic Ocean and the adjacent European continent, and on the diachronous inception of ice caps on either side of the North Atlantic Ocean. This pattern agrees well with a drop in sea level during the Eemian while the contemporaneous climate in western Norway probably inhibited the growth of any large ice-masses in Scandinavia[46]. A marked cooling of sea-surface temperatures, which occurred in the western North Atlantic and in the North Pacific[61], while both the eastern North Atlantic and the adjacent European continent remained warm, could probably be regarded as a natural trend which might have later promoted the transition to full glacial conditions in the Northern Hemisphere.

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Reverse gyrase—a topoisomerase which introduces positive superhelical turns into DNA

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An enzymatic activity which converts closed circular DNA into a positively supercoiled form is present in cellular extracts of Sulfolobus, *an acidothermophilic archaebacterium. This novel enzyme is a type II DNA topoisomerase and is active only at temperatures > 55 °C, in the presence of ATP and* Mg^{2+}.

THE topological state of DNA[1] affects its replication[2,3], recombination[4–6], gene expression mediated by transcription[7,8] and nucleosome assembly[9–11]. DNA topoisomerases have been detected in a variety of organisms[1] and have been characterized and classified into two categories: type I topoisomerases transiently cut one of the two DNA strands to change the linking number in steps of one and type II topoisomerases act by means of a transient double-stranded break, which results in changing the linking number by two steps at a time. In eukaryotic cells, the type I enzyme, the so-called 'nicking–closing' enzyme, is predominant. This enzyme acts on both negatively and positively supercoiled DNAs, removing their superhelical turns. An ATP-requiring type II topoisomerase was first discovered by its particular ability to catenate and decatenate[12,13] or to unknot the entangled DNA rings[14,15]. This type II enzyme can also remove both negative and positive superhelical turns from DNA. In a typical bacterial extract, two types of topoisomerases are detected: ω-protein (type I) which relaxes negatively but not positively supercoiled DNA, and DNA gyrase (type II) which introduces negative superhelical turns into DNA[16].

Archaebacteria are believed to be a third type of organism[17] which have various characteristics distinct from those of eukaryotes or prokaryotes. We surveyed topoisomerase activities in extracts of *Sulfolobus*, an acidothermophilic archaebacterium, and found two remarkable features. First, in contrast to other organisms, ATP-requiring type II topoisomerase activities prevailed and were easily fractionated into three different enzymatic preparations, while type I topoisomerase activity was negligibly small. Second, one of the type II topoisomerases was able to introduce positive superhelical turns into DNA. We have tentatively named this new enzyme reverse gyrase and discuss possible implications of its biological function.

Identification of topoisomerases

Topoisomerase activities were surveyed in the following four conditions: (1) Relaxation of negatively supercoiled DNA was measured in the presence of EDTA, to detect eukaryotic type I nicking–closing activity. (2) The same conditions as for condition (1) but Mg^{2+} was added instead of EDTA, to detect ω-like activity (type I). (3) The same conditions as in condition (2) except that 1 mM ATP was included (type II). (4) Supercoiling of relaxed DNA was assayed in the presence of ATP and Mg^{2+}, to detect a gyrase-like activity (type II).

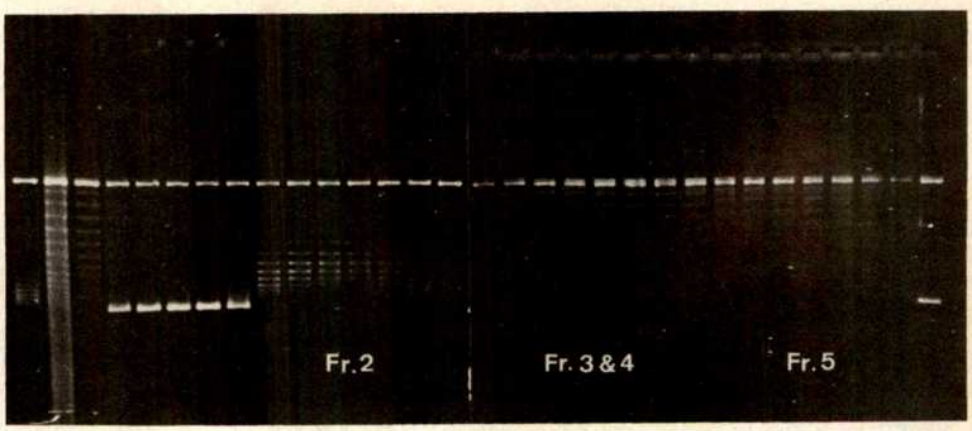


Fig. 1 Fractionation of topoisomerase activities. Lane 1, the activity in the crude extract; all the other lanes show the activity in each fraction of the column.

Methods: The *Sulfolobus acidocaldarius* strain 7 (FERM P-7137, Fermentation Research Institute, Japan) used in this study was similar but not identical to *S. acidocaldarius* strain DSM 639 (ref. 32). The antiserum raised against pure RNA polymerase of the strain DSM 639 (provided by Dr W. Zillig) cross-reacted with that of our strain 7, although the molecular weights of each subunit examined by SDS-polyacrylamide gel electrophoresis were not identical between the two strains (unpublished results). Cells were grown at 75 °C for 24 h with aeration in medium containing 0.1% yeast extract, 0.1% polypeptone, 0.1% casamino acids, 0.1% glucose, 0.2 g l^{-1} NaCl, 1.3 g l^{-1} $(NH_4)_2SO_4$, 0.3 g l^{-1} KH_2PO_4, 0.25 g l^{-1} $MgSO_4$, 0.07 g l^{-1} $CaCl_2$ and 2.5–5 ml 1M H_2SO_4 to adjust the *p*H to 3.0. About 1.7 g (wet weight) of cells obtained from 2 l of culture were suspended in 50 mM Tris-HCl *p*H 7.5, 10% sucrose, 1 mM EDTA–Na_3 and 10 mM β-mercaptoethanol and disrupted by three cycles of freeze-thawing. Two successive centrifugations were done, first at 12,000 r.p.m. for 30 min to remove cell debris, then at 40,000 r.p.m. for 2 h to obtain a clear supernatant (crude extract). The crude extract was adsorbed to a DEAE–Sephacel column (1.2 × 6 cm) in the same buffer as above except that 10% glycerol was used instead of 10% sucrose, then fractionated with a linear gradient from 0.025 M to 0.5 M NaCl. Topoisomerase activity in each fraction was measured in a standard assay mixture (35 mM Tris-HCl *p*H 7.5, 1.8 mM spermidine-$(HCl)_3$, 0.14 mM EDTA–Na_3, 5 mM dithiothreitol, 24 mM KCl, 10 mM $MgCl_2$, 1 mM ATP, 90 μg ml^{-1} tRNA and 8.7 μg ml^{-1} of negatively supercoiled pBR322 DNA as a substrate) at 65 °C for 1 h. The reaction was stopped by adding 1% SDS, 10 mM EDTA–Na_3 and 5% glycerol, and the relaxation in superhelicity of pBR322 DNA was monitored by 1% agarose gel electrophoresis at room temperature in 89 mM Tris-base, 90 mM boric acid, 2.5 mM EDTA–Na_3. A ladder of DNA bands, each representing one topoisomer, was visualized after gels were stained in 0.5 μg ml^{-1} EtBr. The three peak fractions of relaxing activities shown here were pooled separately and adsorbed to a phosphocellulose column (0.6 × 5.5 cm) equilibrated with 20 mM K-phosphate *p*H 7.0, 10 mM β-mercaptoethanol, 0.1 mM EDTA–Na_3 and 10% glycerol. A linear gradient of NaCl (0–1.0 M) was applied to recover the topoisomerase activities. The active fractions Fr.2, Fr.3, Fr.4 and Fr.5 (described in Table 1) were dialysed against 50 mM Tris-HCl *p*H 7.5, 0.1 mM EDTA–Na_3, 10 mM β-mercaptoethanol and 50% glycerol. All steps were carried out at room temperature. Each fraction was stored at −20 °C and remained active for 2 yr.

Table 1 Properties of the topoisomerases found in *Sulfolobus*

	DEAE–Sephacel (NaCl, M)	Phospho-cellulose (NaCl, M)	MW × 10^3	Require-ment	Relaxation of negative super-coil	Relaxation of positive super-coil	Unknot-ting	Catena-tion	Super-coiling	Linking number change
Fr.2	0.1–0.15	0.4–0.5	200	Mg, ATP	+ + +	+ + +	+	+ + +	+ +	2
Fr.3	0.15–0.3	0	330	Mg	+ +	−	−	−	−	1
Fr.4	0.15–0.3	0.6–0.7	500	Mg, ATP	+ + +	−	+	+	+ + +	2
Fr.5	0.3–0.5	0	460	Mg, ATP	+ + +	+ + +	+ + +	−	−	2

The molecular weight (MW) of each topoisomerase activity was estimated by sedimentation in a 20–40% glycerol gradient at 45,000 r.p.m. for 20 h with the following internal markers: β-galactosidase, bovine serum albumin, haemoglobin and cytochrome *c*. The relaxation of positively supercoiled DNA was tested by adding excess EtBr to the standard assay mixture (see Fig. 1 legend). EtBr was extracted with *n*-butanol before agarose gel electrophoresis. The product of Fr.4 enzyme was also used as a positively supercoiled substrate. Unknotting activity[14] was detected in the standard assay condition at 65 °C using knotted DNA extracted from P4 defective (tail-less) particles. A DNA band appeared at the position corresponding to the size of the P4 relaxed circle in the presence of ATP, whereas without enzyme or ATP no discrete bands were obtained. When incubation was at 75 °C, knotted DNA was pretreated with T4 DNA ligase to seal nicks at the cohesive ends[15], otherwise a non-enzymatic reaction occurred. Catenating activity[12,13] was judged from the DNA remaining at the top of the gel (see Fig. 3*c*, lane 4, for example), which was visualized as large aggregates under the electron microscope. Decatenating activity was measured by using these aggregates as a substrate.

As *Sulfolobus* grows at ~75 °C and not at temperatures <60 °C, incubation was at 65–75 °C for 1 h, after which the reaction was stopped by adding EDTA and SDS at room temperature, and the change in the linking number of closed circular DNA was monitored by agarose gel electrophoresis. No enzymatic activity in the crude extract was evident in conditions (1), (2) or (4), whereas a small amount of activity was detected in condition (3).

The crude extract was fractionated by DEAE–Sephacel chromatography. Figure 1 shows that activities in condition (3) above which relaxed negatively supercoiled DNA were detected in three separated fractions (Fr.). Each peak fraction was pooled and applied to a phosphocellulose column for further purification. The first peak and the third peak of the DEAE–Sephacel column exhibited single activities (Fr.2 and Fr.5), both requiring ATP. The second peak was divided into two fractions, one (Fr.3) active in the absence of ATP and the other (Fr.4) requiring ATP. The relaxation activity of each fraction sedimented at a unique position on glycerol gradient centrifugation, from which the molecular weight of each enzyme was estimated. Other activities such as supercoiling, unknotting of knotted P4 DNA[14,15] and catenation[12,13,15], were assayed for each enzyme preparation. The activity in Fr.4 is the main subject of this paper. The characteristics of the other topoisomerases are briefly summarized in Table 1 and will be reported in detail elsewhere.

Staggered ladders of DNA topoisomers

The enzymatic activity in partially purified Fr.4 was absolutely dependent on the addition of Mg^{2+} and ATP (Fig. 2*a*), and was demonstrably a type II topoisomerase[16]. The Fr.4 enzyme relaxed a DNA of unique negative superhelicity by two linking number changes at a time (Fig. 2*b*, lane 3). When relaxed DNA was used as a substrate, Fr.4 was able to introduce superhelical turns into DNA (Fig. 2*c*), showing ladders (a set of DNA topoisomers with different superhelicities) migrating more rapidly on agarose gel electrophoresis[17]. However, this gyrase-like activity was not evident at all with a DNA substrate of a defined negative superhelical density. Figure 2*b*, lane 3, shows that the Fr.4 enzyme could not negatively supercoil this substrate further but only relax it. By contrast, a more negatively supercoiled band was formed by the Fr.2 enzyme (Fig. 2*b*, lane 4), which served as a control for the gyrase activity. Furthermore, the position of each band of the ladders formed by the Fr.4 enzyme was slightly different and staggered compared with the

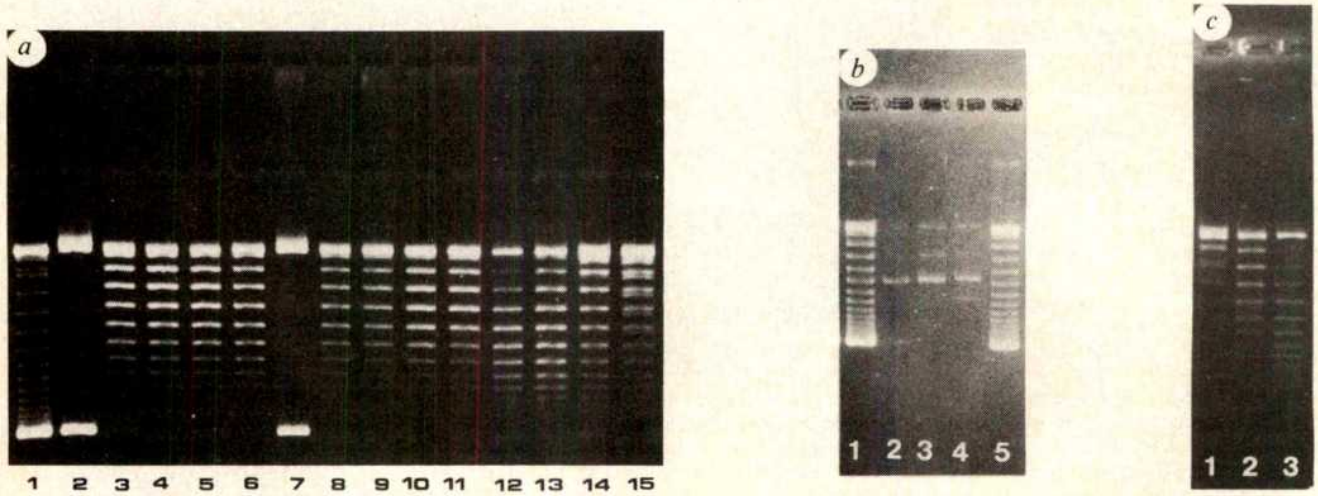


Fig. 2 Topoisomerase activity in Fr.4. *a*, Determination of the concentrations of Mg^{2+}, ATP and KCl optimal for Fr.4 activity. Each component was omitted from the standard assay mixture and then various amounts were added as indicated. Lane 1, pBR322 (negatively supercoiled topoisomers). Lanes 2–6, effect of Mg^{2+} concentration (0, 5, 10, 15 and 20 mM added in lanes 2–6, respectively). Lanes 7–11, effect of ATP concentration (0, 0.1, 0.2, 1 and 2 mM, respectively). Lanes 12–15, effect of KCl concentration (0, 50, 100 and 150 mM, respectively). *b*, Change of the linking number. A DNA band corresponding to a unique negative superhelicity was extracted from the ladder in agarose gel, and incubated with Fr.4 in conditions chosen to produce as minimal a change in linking number as possible. pBR322 DNA with a unique superhelical density (lane 2) was incubated with Fr.4 (lane 3) or Fr.2 (lane 4). The Fr.2 enzyme serves as a control demonstrating the linking number change by +2 and −2. Lanes 1 and 5 represent markers of negatively supercoiled topoisomers of pBR322. *c*, Supercoiling activity. Relaxed pBR322 DNA (lane 1) was used as a substrate in the standard condition and was incubated with Fr.4 at 65 °C (lane 2) or 75 °C (lane 3).

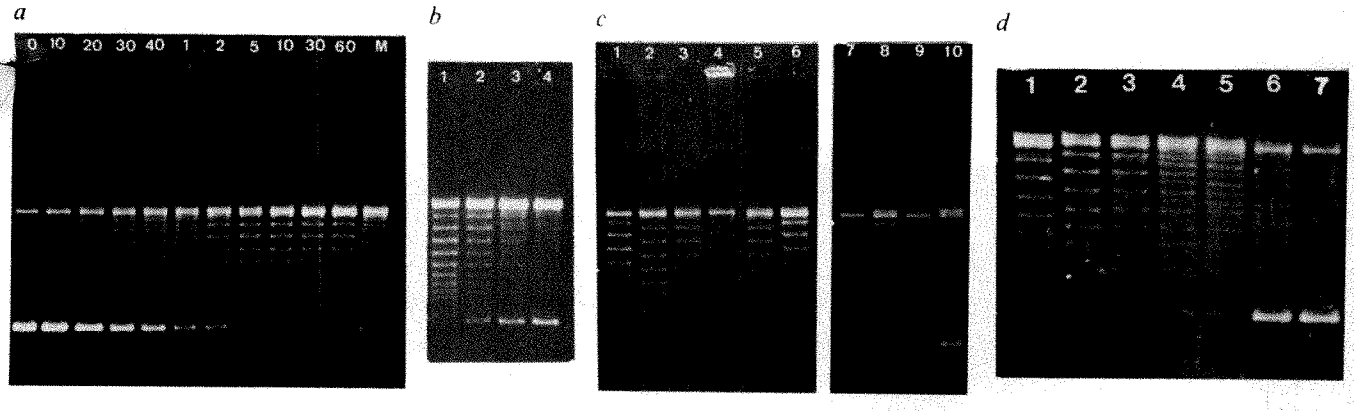


Fig. 3 Staggered ladders formed by Fr.4 enzyme. *a*, The time course of Fr.4 activity was monitored in standard assay conditions using 0.3 μg of Fr.4 protein and 0.26 μg of negatively supercoiled pBR322 DNA as a substrate. The reaction was stopped at the time indicated at the top of the column: 0, 10, 20, 30, 40 s, then 1, 2, 5, 10, 30, 60 min. The lane labelled M shows negatively supercoiled topoisomers of pBR322 prepared by yeast nicking–closing enzyme. *b*, Fr.4 activity was examined by changing the enzyme concentration. The amount of protein in each reaction mixture was (lanes 1–4, respectively): 0.15 μg, 0.03 μg, 0.015 μg and 0.003 μg. *c*, Interconversion of staggered ladders by other topoisomerases. Lane 1, a set of negatively supercoiled pBR322 topoisomers prepared with Fr.2 (standard ladder). Lane 2, Fr.4 was added to the sample prepared as in lane 1 and incubated further. The ladders were staggered. Lane 3, the reaction product of Fr.4 (staggered ladders). Lane 4, Fr.2 was added to lane 3 and incubated further. The ladder converted to the standard one and the catenated DNA also. Lane 5, same as lane 3. Lane 6, Fr.5 was added to lane 5 and incubated further. Lane 7, same as lane 3. Lane 8, yeast nicking–closing enzyme was added to lane 7 for further incubation at 30 °C. Lane 9, *E. coli* ω-protein was added to lane 7 for further incubation at 37 °C. Lane 10, a set of negatively supercoiled pBR322 topoisomers prepared by yeast nicking–closing enzyme (standard ladder). *d*, Catalytic nature of the enzyme. pBR322 DNA (0.26 μg) was included in each reaction mixture, while the amount of protein in the purified enzyme fraction was (lanes 2 to 7, respectively) 0.012 μg, 0.006 μg, 0.003 μg, 0.0015 μg, 0.0006 μg and 0.0003 μg. Lane 1 serves as a marker of negatively supercoiled topoisomers of pBR322 prepared by yeast nicking–closing enzyme (at 30 °C for 10 min). Note that a nicked circle, a relaxed circle, and supercoils with one positive turn and with one negative turn overlap with the two topmost bands of the ladders. The marker (lane 1) is rich in negatively supercoiled (−1 turn) and relaxed circles, while many nicked circles are found in the sample incubated at 75 °C (lanes 2–7). This explains a slight difference in the position of the top band in lane 1.
Methods: *d*, Fr.4 was further purified on a hydroxylapatite column equilibrated with 0.1M phosphate buffer (*p*H 7.0) containing 0.25 M NaCl. The topoisomerase activity was eluted by a linear gradient of phosphate (0.1–1.0 M). An active fraction recovered at 0.35 M phosphate was used for this experiment. The protein concentration was measured using Bio-Rad protein assay reagents. As the moecular weight of the enzyme was estimated to be ~500,000 (close to the β-galactosidase marker in glycerol gradient centrifugation), the molar ratio of protein to pBR322 DNA was calculated to be (lanes 2–7, respectively): 0.24, 0.12, 0.06, 0.03, 0.012 and 0.006.

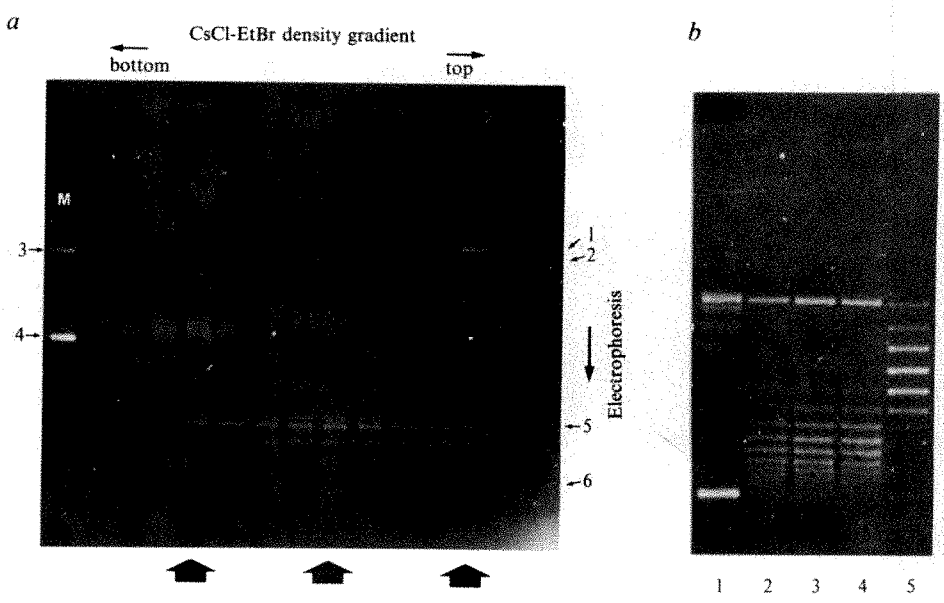


Fig. 4 CsCl–EtBr density gradient centrifugation. *a*, The first lane (indicated by M) is a size marker for pBR322 DNA. All the other lanes correspond to fractions from the CsCl–EtBr density gradient. Only an appropriate part of the gel is shown, where covalently closed circular DNA is present. The numbers indicate the positions of the following DNAs: (1) dimeric pBR322 (negatively supercoiled), (2) pBR322 (nicked circle), (3) pBR322 (relaxed circle), (4) pBR322 (negatively supercoiled), (5) pAO3 (relaxed or nicked circle), (6) pAO3 (negatively supercoiled). *b*, The pattern of the pBR322 DNA in the peak fraction compared with that of standard samples of negatively or positively supercoiled DNA. Lane 1, negatively supercoiled topoisomers of pBR322. Lanes 2–4, peak fractions of positively supercoiled pBR322 shown in *a*. Lane 5, positively supercoiled topoisomers of pBR322.
Methods: *a*, Negatively supercoiled pBR322 (4.3 kilobases, kb) treated with Fr.4 enzyme, was mixed with negatively supercoiled pAO3 (1.8 kb) DNA treated with Fr.5 enzyme (a marker for relaxed DNA at 75 °C) and a negatively supercoiled dimer of pBR322 (8.6 kb) (a marker for negatively supercoiled DNA at 37 °C). The solution was adjusted to 3.7 ml in 10 mM Tris-HCl *p*H 7.8, 10 mM EDTA–Na_3. After adding 3.8 g of solid CsCl and 0.3 ml of 5 mg ml^{-1} EtBr, the mixture was centrifuged in the RTV65 vertical rotor of the Hitachi ultracentrifuge (35,000 r.p.m. at 20 °C for 48 h). The gradient was fractionated from the bottom and DNA was extracted twice with *n*-butanol. After dialysis against 10 mM Tris-HCl *p*H 8, 1 mM EDTA–Na_3, each fraction was anlysed by 1.0% agarose gel electrophoresis. *b*, Positively supercoiled topoisomers were prepared by incubating negatively supercoiled pBR322 DNA with yeast nicking–closing enzyme at 4 °C for 24 h. The resultant molecules were positively supercoiled when assayed at 24 °C (ref. 19).

negatively supercoiled topoisomers prepared by yeast nicking–closing enzyme (Fig. 2*a*, compare lane 1 with the rest).

In favourable conditions of gel electrophoresis, the staggered bands formed by Fr.4 enzyme migrated just between the standard array of negatively supercoiled topoisomers, prepared either by yeast nicking–closing enzyme or Fr.2 or Fr.5 enzyme (compare Fig. 3*c*, for example). The time course of the reaction with Fr.4 enzyme (see Fig. 3*a*) demonstrated that in a short incubation time, two sets of the ladders, regular and staggered, appeared and as time progressed, the former bands disappeared and the latter ones intensified. A similar pattern was observed with decreasing amounts of enzyme, where doublet ladders were evident (see Fig. 3*b*). These results suggested that staggered ladders were formed via standard ladders when the reaction proceeded further. The pattern of the staggered ladder was not affected by further deproteinization with phenol and chloroform–isoamyl alcohol. It converted to a normal (negatively supercoiled) ladder after treatment with Fr.2 enzyme, which contained DNA gyrase and DNA catenase activity (Fig. 3*c*, lanes 3, 4), or with Fr.5 enzyme, a type II topoisomerase which could relax both negative and positive supercoils (Fig. 3*c*, lanes 5, 6). Therefore, this staggered ladder must be attributed to the DNA topoisomers. The reaction product of Fr.4 enzyme was next treated with either yeast nicking–closing enzyme or *Escherichia coli* ω-protein. The former catalysed the relaxation of staggered ladders but the latter could not (Fig. 3*c*, lanes 7–9). As distinct from the yeast enzyme, ω-protein does not relax positively supercoiled DNA. This result suggests that the staggered ladders comprise positively supercoiled DNA topoisomers.

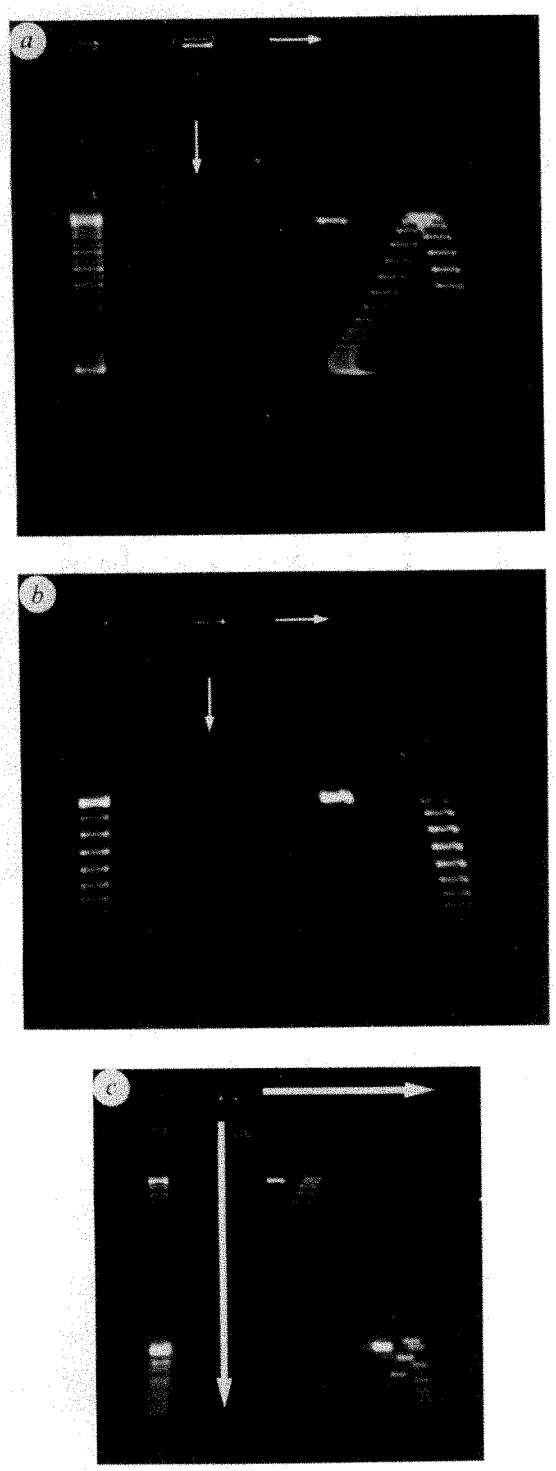


Fig. 5 Two-dimensional agarose gel electrophoresis. *a*, A mixture of negatively supercoiled pBR322 and positively supercoiled pBR322 prepared as described for Fig. 4*b* was electrophoresed in the first dimension in the standard conditions. The gel was soaked in 0.02 μg ml^{-1} EtBr solution for 90 min and re-electrophoresed in the orthogonal direction in the same buffer containing 0.02 μg ml^{-1} EtBr. *b*, pBR322 DNA treated with Fr.4 enzyme was electrophoresed in the same way as for *a*. *c*, A mixture of pBR322 treated with Fr.5 enzyme and pAO3 treated with Fr.4 enzyme was analysed as described above.

Positively supercoiled topoisomers

To distinguish between the various possible conformations of DNA, the reaction product of Fr.4 was centrifuged in a CsCl equilibrium density gradient in the presence of ethidium bromide (EtBr) then each fraction was analysed by agarose gel electrophoresis. Figure 4*a* shows that pBR322 DNA incubated with Fr.4 enzyme formed a band of much heavier density than the relaxed form of pAO3 DNA[18] (prepared by Fr.5 enzyme) which was used as an internal marker, indicating that the former represented positively supercoiled DNA. The ladders at the peak position of pBR322 (Fig. 4*b*, lanes 2–4) coincided with a standard sample of positively supercoiled topoisomers (lane 5)[19] but not negatively supercoiled ones (lane 1).

Two-dimensional agarose gel electrophoresis[20] provides another means of distinguishing between positive and negative superhelical DNA topoisomers. After electrophoresis in the first dimension, gels were soaked in a mild concentration of EtBr and electrophoresed in the orthogonal direction. In the second dimension, positively supercoiled DNA had a higher mobility due to the increase in the superhelical density by EtBr, while the migration of negatively supercoiled DNA was retarded because EtBr relaxed it. Figure 5*a* shows clear separation of a control mixture of negatively and positively supercoiled DNA topoisomers in the second dimension, forming a symmetrical arch pattern. The reaction products of Fr.4 enzyme (Fig. 5*b*) migrated more rapidly in the second dimension, indicating that they were in fact positively supercoiled DNA. In Fig. 5*c*, a mixture of pAO3 incubated with Fr.4 enzyme and pBR322 treated with Fr.5 enzyme (a control for relaxed DNA at 75 °C) was developed by two-dimensional agarose gel electrophoresis. The pattern of pAO3 clearly showed the formation of positively supercoiled DNA topoisomers.

Superhelicity at high temperature

It should be noted that our reaction temperature was 75 °C and that the products were analysed at 24 °C. The superhelicity of DNA is known to be affected by temperature[19]. Thus, in the conditions of our analysis, the DNA would be less positively supercoiled, or more negatively supercoiled, than in the reaction mixture. The difference in superhelicity caused by temperature was roughly three superhelical turns per 20 °C in our system using pBR322 (data not shown). Note also that the fully relaxed pBR322 DNA formed by the topoisomerase in Fr.5 at 75 °C (see Table 1) appeared to be negatively supercoiled by five turns in our assay conditions. DNA must be more positively supercoiled by several turns in our reaction mixture at 75 °C than was actually observed at the ambient temperature.

Reverse gyrase—a novel enzyme?

The reverse gyrase studied here might consist of two components: a putative DNA-binding protein which introduces positive superhelical constraints, consequently creating negatively supercoiled domains in the unoccupied parts of closed circular DNA, and a topoisomerase which could relax these regions. The resulting DNA would then be positively supercoiled on removal of the binding proteins. This type of mechanism was excluded by the following two experiments.

First, the minimal protein concentration required for reverse gyrase activity (for this purpose, Fr.4 was further purified by hydroxylapatite chromatography) was estimated to be less than one-tenth the molar ratio of the substrate DNA (Fig. 3*d*). This amount of protein in the reaction mixture is insufficient to constitute a nucleosome-like complex, as DNA-binding proteins would be needed in a molar excess over the DNA. A preliminary analysis of protein components in this fraction by SDS-polyacrylamide gel electrophoresis revealed four major bands in equimolar amounts (molecular weights 124,000, 101,000, 45,000 and 42,000). Second, the linking number of DNA in the hypothetical protein–DNA complex should not be changed by adding exogenous topoisomerases as the DNA should be relaxed before deproteinization, while free positively supercoiled DNA should be a good substrate for the second topoisomerase. This notion was tested by further incubation of the reaction product of Fr.4 with Fr.5 enzyme at 75 °C or with yeast nicking–closing enzyme at 30 °C. Figure 3*c*, lanes 5–8 shows that the final products were relaxed DNAs, indicating that the positively supercoiled DNA was not complexed in the reaction mixture. Therefore, the Fr.4 activity was not due to a combination of DNA-binding proteins and a topoisomerase, but Fr.4 must contain the unique enzymatic activity of a reverse gyrase which produces DNA with positive superhelical tension.

In living organisms, no positively supercoiled DNA has ever been reported, nor has an enzyme been found to produce such a topological constraint. It is possible that positive supercoils are relaxed as soon as they are formed, by the potent type I topoisomerases generally found in eukaryotic cells. In *Sulfolobus*, type I topoisomerase activity was low compared with the three other topoisomerases (see Table 1), which may have been essential for our discovery of the novel reverse gyrase activity. Positively supercoiled DNA can be prepared *in vitro* by: (1) using intercalating drugs such as EtBr; (2) raising the temperature of a solution containing relaxed circular DNA prepared at a lower temperature; (3) binding large amounts of DNA gyrase to nicked DNA, then sealing it and removing the proteins[21]; (4) using stoichiometric amounts of DNA topoisomerase II'[22]; or (5) using netropsin and a DNA topoisomerase[23]. Here, we have shown that reverse gyrase can form positively supercoiled DNA by changing the linking number of closed circular DNA. The biological activity of such a DNA molecule is of great interest, as well as its physicochemical properties.

Compared with the extent of the positive superhelicity produced by intercalating pBR322 DNA with EtBr (more than 20 turns), the superhelicity produced by reverse gyrase appeared incomplete. We observed positively supercoiled DNA with eight turns in our gel analysis at 25 °C (see Fig. 3*c*, lane 7 and Fig. 4*b*, lane 3, for example), thus we estimate 13 positive superhelical turns at best in our reaction at 75 °C. There are two possible explanations for this: first, there is an upper limit to increasing the linking number of DNA because of some physical stress in the DNA helix itself. The positively supercoiled circles formed by netropsin binding, described above, represent the same situation[24]. Second, as the DNA becomes more positively supercoiled, so its ability to react with the enzyme might decrease.

Why is reverse gyrase needed?

Negatively supercoiled DNA is believed to be a favourable substrate for replication, transcription and recombination. Conversely, positively supercoiled DNA might be unfavourable for these functions. So what is the role of a topoisomerase which introduces positive superhelical strains into DNA? One trivial explanation is that because *Sulfolobus* grows at 75 °C, reverse gyrase is required to prevent the genomic DNA from denaturation by tightening the helical pitch of DNA (as the G+C content of *Sulfolobus* is only 40%). If this is the case, similar activities should exist in other thermophilic organisms, and their plasmids, if any[25]; no such examples have been reported.

We suggest, on the other hand, that reverse gyrase is distributed widely in many organisms to counteract the effects of other topoisomerases. It may actively alter the superhelicity of DNA so as to disassemble the stable complexes of proteins and negatively supercoiled DNA. For example, chromatin DNA is held in a stable complex with histones but it is believed that DNA must be freed from histones for replication, recombination, transcription, etc. To release the histones from nucleosomes it may be necessary to reverse the assembly process[26], in which positive superhelical turns have been removed by topoisomerases. Instead, the reverse gyrase could introduce positive superhelical turns into the internucleosomal regions and the resulting superhelical strain could detach the histones from the DNA.

A similar situation may prevail when cruciform structures or segments of Z-DNA[27] that are formed in the negatively supercoiled DNA[28,29] need to be reorganized to regular B-form DNA. Relaxing the negatively supercoiled DNA may not provide sufficient driving force to cause structural changes (probably because specific proteins must be associated) and here reverse gyrase may be needed. The transition of such structures in chromosomes may be important as initiation signals for DNA replication, recombination or gene expression[30,31].

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Use of light for footprinting DNA *in vivo*

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A new 'photofootprinting' technique, which uses light to detect protein–DNA contacts as well as changes in the structure of DNA at the base pair level, has been developed and used to detect contacts between lac *repressor and the* lac *operator in* Escherichia coli *cells.*

ALTHOUGH it is well established that interactions between DNA and protein regulate gene expression, there are few techniques capable of directly probing these interactions *in vivo*. Given the complexity of the intracellular environment and our inability to reconstruct it, accurate molecular descriptions of gene regulation are critically dependent on the development of such techniques. Currently, experiments using DNase I 'footprinting'[1], chemical modification[2,3], bromouracil cross-linking[4] and drug cleavage[5,6] can probe, with high resolution, protein–DNA interactions *in vitro*. In each technique a protein is bound to a uniquely end-labelled DNA fragment. The complex is then subjected to an enzymatic, chemical or photochemical procedure which either breaks the DNA backbone directly or modifies the DNA in such a way that its backbone can be subsequently broken by alkali[7]. After removal of protein, the labelled DNA is denatured and electrophoresed in a polyacrylamide sequencing gel. An autoradiograph of this gel shows a pattern of end-labelled fragments which correspond to breaks in the DNA backbone induced by the modification or cleavage procedure. As cleavage rates may be altered by protein binding, corresponding changes in the strand breakage pattern may be used to identify protein contacts.

Although protein binding sites can be identified and characterized *in vitro* by these techniques, the chemical or enzymatic procedures used to induce strand cleavage are often too harsh and too slow to be used on living cells without perturbing native protein–DNA interactions. We report here the development of a new technique, termed 'photofootprinting', which uses light to probe protein–DNA interactions *in vitro* and *in vivo*. The technique, in its simplest form, is based on the observation that a small but significant distortion of the double helix is necessary to form a UV photoproduct[8–11]. If protein contacts affect the geometrical changes necessary for photoproduct formation, exposure of protein-free and protein-bound DNA to light followed by breakage of the DNA backbone only at the sites of photodamage will yield two different sets of DNA fragments. When analysed on a sequencing gel, differences in the strand-breakage pattern of these two DNAs will occur at those bases in contact with protein. More elaborate procedures based on photochemical reactions between DNA and small probes such as photosensitizers will be described below.

To detect protein–DNA contacts using light, we have developed a chemical procedure which exploits a common feature of photoreacted bases absent in unmodified bases so as to break the DNA backbone only at the sites of photodamage. In each of the known major photoproducts in DNA, one or more of the 5,6-double bonds of a pyrimidine becomes saturated on photoreaction[8,12]. As carbonyl groups conjugated to a double bond are poorly reduced by sodium borohydride[13], the carbonyl group at position 4 of photochemically modified thymines can be selectively reduced in the presence of other bases[14]. Furthermore, Kunieda and Witkop[15] and Miller and Cerutti[14,16], have shown that reduction of thymines saturated at the 5,6-position with excess $NaBH_4$ opens the thymine ring. As conjugation of the lone pair electrons of the glycosidic nitrogen at position 1 is greatly reduced by saturation of the 5,6-double bond and ring opening, the glycosidic bond of photochemically modified and reduced thymine should be much more labile to acid hydrolysis than unmodified thymine. Once the glycosidic bond is hydrolysed by acid, the phosphate backbone can be broken at the sugar ring by subsequent treatment with alkali[7]. In practice, base hydrolysis and subsequent breakage of the backbone can be carried out in one step by briefly heating in acidic aniline.

Although the 4 position of cytosine is not reduced by $NaBH_4$, saturation of the 5,6-double bond is known greatly to enhance spontaneous deamination of the 4 position, converting it to a carbonyl group[17]. Thus, like thymine, photochemically modified cytosine should be selectively reduced and its ring opened by $NaBH_4$, labilizing its glycosidic bond to acid hydrolysis and subsequent strand breakage.

Photofootprinting the *lac* repressor *in vitro*

To determine whether protein contacts can be detected with UV light alone, a 95-base pair (bp) fragment of DNA containing the *lac* operator[18] was exposed for 20 and 80 s to a 150-W xenon lamp in the presence and absence of *lac* repressor. After purification and after the DNA was end-labelled with ^{32}P, the photoproducts were labilized by the chemical degradation procedure and the resulting strand breakage pattern was analysed on a sequencing gel. In the absence of *lac* repressor, brief irradiation of the *lac* operator induced the formation of many photochemically modified bases which could be detected by the chemical degradation procedure (Fig. 1). Photoproducts were detected not only at pyrimidines but, unexpectedly, also at purines. Although photoreactions between adjacent pyrimidines are well documented[8], photoreactions between pyrimidines and purines are not[12], and further characterization is necessary to establish the identity of these new photoproducts.

When these experiments are repeated in the presence of *lac* repressor, repressor binding inhibited, and in some cases enhanced the ability of base pairs to undergo photochemical modification (Fig. 1). The location of the major repressor contacts is in excellent agreement with the repressor binding site detected by methylation protection[19], DNase I footprinting[20], bromouracil cross-linking[4] and chemical synthesis methods[21]. Thus, brief exposure of the *lac* repressor–*lac* operator complex to UV light can be used to identify repressor contacts.

In contrast to other techniques[19,20], UV light also detects repressor contacts upstream and downstream from the known repressor binding site. Although these weak 'contacts' could result from repressor-induced distortions in adjacent protein-free DNA, we believe them to represent previously unreported or unidentified repressor contacts (see below).

Figure 2 examines in detail the chemistry used to detect the *lac* repressor–*lac* operator photofootprint. Several features of this analysis are noteworthy. First, detection of a photofootprint requires both $NaBH_4$ reduction and acidic aniline cleavage. If either step is excluded, no cleavage of the DNA is observed (compare methods *d* and *e* and *b* and *c*). Second, in the absence of photodamage, cleavage of DNA by the $NaBH_4$–acidic aniline technique is very weak and comparable with the same background cleavage resulting from piperidine treatment (compare the first lanes of methods *a*, *b* and *e*). Thus, the background cleavage of the $NaBH_4$–acidic aniline technique is as low as the background cleavage observed in the chemical sequencing method of Maxam and Gilbert[7]. Third, in these conditions UV light does not directly cleave DNA (method *a*).

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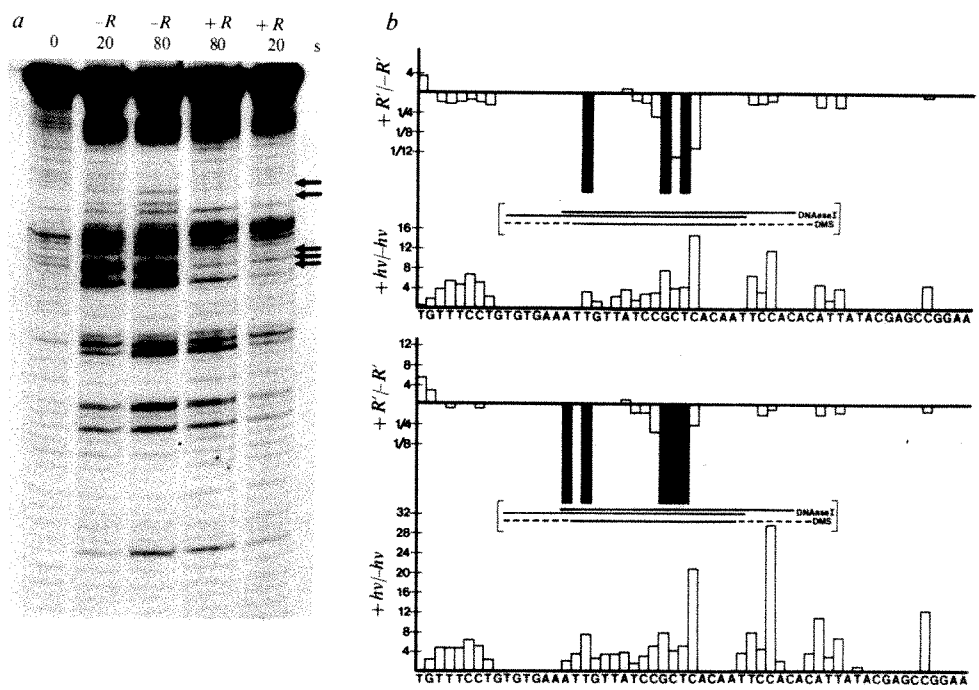


Fig. 1 *In vitro* photofootprints of the *lac* repressor bound to the *lac* operator. *a*, An autoradiogram of the *lac* repressor photofootprint. Lane 1, a no-light control showing the background resulting from the chemical labilization procedure. Lanes 2, 3, the effect of irradiating the *lac* operator for 20 and 80 s, respectively, in the absence of *lac* repressor. Lanes 4, 5, the effect of irradiating the *lac* operator for 80 and 20 s, respectively, in the presence of *lac* repressor. The level of photodamage in these experiments is very low: ~1 bp in 500 is damaged by light. Arrows denote bases whose photodamage is totally blocked by repressor binding. *b*, results of the autoradiogram quantified by microdensitometry and displayed as a histogram. The upper half summarizes the results of the 20-s irradiation experiment and the lower half those of the 80-s irradiation experiment. The effect of light on the cleavage of DNA in the absence of *lac* repressor is expressed as the ratio $(+h\nu/-h\nu)$, where $+h\nu$ is the cleavage of a base in the presence of light and $-h\nu$ is its cleavage in the absence of light. The effect of added *lac* repressor on light-induced cleavage of a base is expressed as the ratio $(+R'/-R')$ where $+R'$ is the light-induced cleavage of a base in the presence of *lac* repressor minus its cleavage in the absence of light and $-R'$ is the cleavage of the base in the absence of repressor minus its cleavage in the absence of light. When the value of $(+R'/-R')$ is $<1/20$, protein binding is considered to block photodamage totally and the corresponding position in the histogram is blackened. In brackets, that region of the *lac* operon protected by *lac* repressor from DNase I digestion is shown as a pair of lines[20]. The upper line summarizes the protection results for the same DNA strand analysed in the photofootprinting experiments and the lower line summarizes the results for the opposite strand. That region of the *lac* operator protected by *lac* repressor from DMS is shown below the DNase I results[19]. Strong protection is denoted by a continuous line and weak protection by a dashed line.

Methods: Irradiation was conducted with a 150-W xenon lamp (PRA model ALH215) equipped with an elliptical mirror. The output of the lamp consists of a focused beam 3 mm in diameter which is passed through a 10-cm long water filter to remove IR radiation before impinging on the sample. Samples were irradiated in 200 μl Micropets (Clay Adams) sealed at one end with a rubber septum. Although the Micropets absorb a small amount of UV radiation, similar results were obtained in UV-transparent cuvettes. In a typical experiment, 12 μl of a solution consisting of 10 mM Tris (*p*H 7.4), 10 mM $Mg(OAc)_2$, 10 mM KCl, 0.1 mM EDTA, 0.1 mM 2-mercaptoethanol, 12% glycerol, 100 μg ml^{-1} gelatin, 15 ng of a 95-bp *lac* operator fragment[18] and 0.1 μg of pBR322 plasmid DNA was irradiated in the absence or presence of 1.4 μg of *lac* repressor. 10 μg of carrier tRNA was then added to each sample and repressor removed by phenol extraction. The *Eco*RI end of the *lac* operator fragment was labelled with 25 μCi of [α-^{32}P]dATP using the Klenow fragment of *E. coli* DNA polymerase I and unreacted ATP was removed by dialysis against 2 M NaCl, 1 mM Tris (*p*H 7), followed by dialysis against 1 mM Tris (*p*H 7). The solutions were transferred to a 1.5-ml Eppendorf tube and heated at 50 °C for 5 h in 1 mM Tris (*p*H 7), 5 mM NaCl to effect deamination of photochemically modified cytosines. After precipitation from ethanol, each sample was dissolved in 50 μl of 5 mM KP_i (*p*H 8.3), 5 mM NaCl containing 50 mM thymidine and cooled to 0 °C. 50 μl of the same KP_i/NaCl/thymidine solution containing 1 mg of freshly dissolved sodium borohydride was then added. The samples were briefly vortexed and allowed to stand unagitated and open to the air at 2 °C in the dark. Thymidine is present during the reduction step to react with any borane generated by the decomposition of $NaBH_4$ in water. Otherwise, borane, which preferentially reduces carbonyl groups conjugated to double bonds, would reduce those bases that are not damaged by light. The reactions were stopped after 15 h by adding 250 μl of 0.7 mM NaOAc–HOAc (*p*H 5.0) and allowed to stand at room temperature for 1 h with occasional vortexing. After precipitating the DNA from 750 μl of ethanol the dried and rinsed pellets were redissolved in 170 μl of 0.5 M NaOAC–HOAc (*p*H 5.0). Following incubation at room temperature for 1 h with occasional vortexing, 100 μl of water and 750 μl of ethanol were added to precipitate the DNA. The rinsed and dried DNA pellets were then dissolved in 2 μl of water and 25 μl of 1.0 M aniline–HOAc (*p*H 4.5) was added[39,40]. After heating at 60 °C for 20 min in the dark, the samples were frozen at −78 °C and lyophilized under high vacuum. Freezing and lyophilization from 25 μl of water was repeated three more times and the DNA twice from ethanol. Following rinsing and drying of the DNA pellet, the samples were dissolved in 98% deionized formamide, boiled for 3 min and electrophoresed on a 20% polyacrylamide sequencing gel at 1,500 V for 14 h.

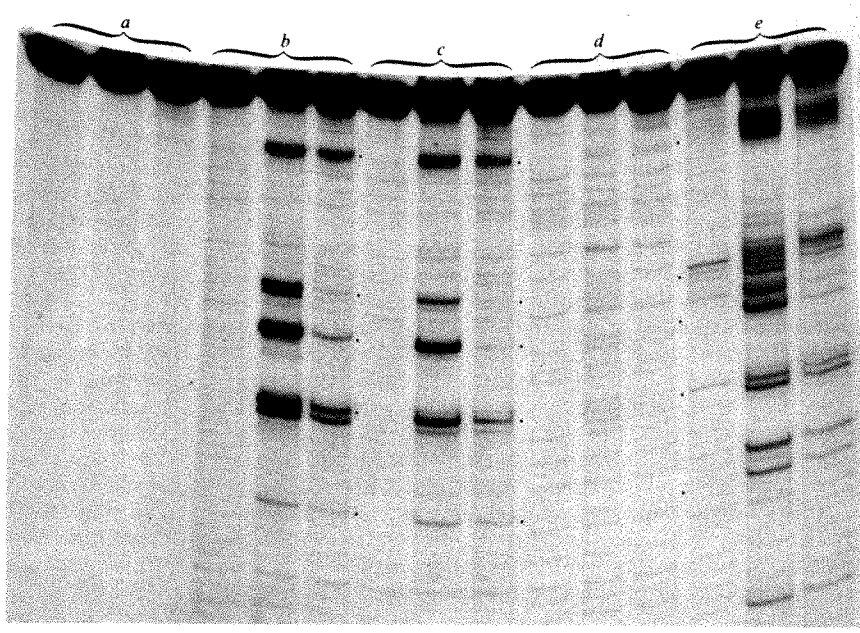


Fig. 2 *In vitro* photofootprints of the *lac* repressor bound to the *lac* operator detected by different chemical treatments (*a*–*e*). For each chemical treatment, three lanes are shown. The first lane of each set is a no-light control, the second lane shows the effect of irradiating the *lac* operator for 40 s in the absence of *lac* repressor and the third lane shows the effect of irradiating the *lac* operator for 40 s in the presence of *lac* repressor. *a*, No chemical treatment; *b*, cleavage is induced by heating in piperidine; *c*, cleavage is induced by successively deaminating, reducing with $NaBH_4$ and heating in piperidine; *d*, cleavage is induced by successively deaminating and heating in acidic aniline; *e*, cleavage is induced by successively deaminating, reducing with $NaBH_4$ and cleaving with acidic aniline.

Methods: DNA was cleaved with piperidine by dissolving the DNA in 100 μl of freshly diluted 1 M piperidine and heating at 90 °C for 25 min[7].

All other methods are identical to Fig. 1.

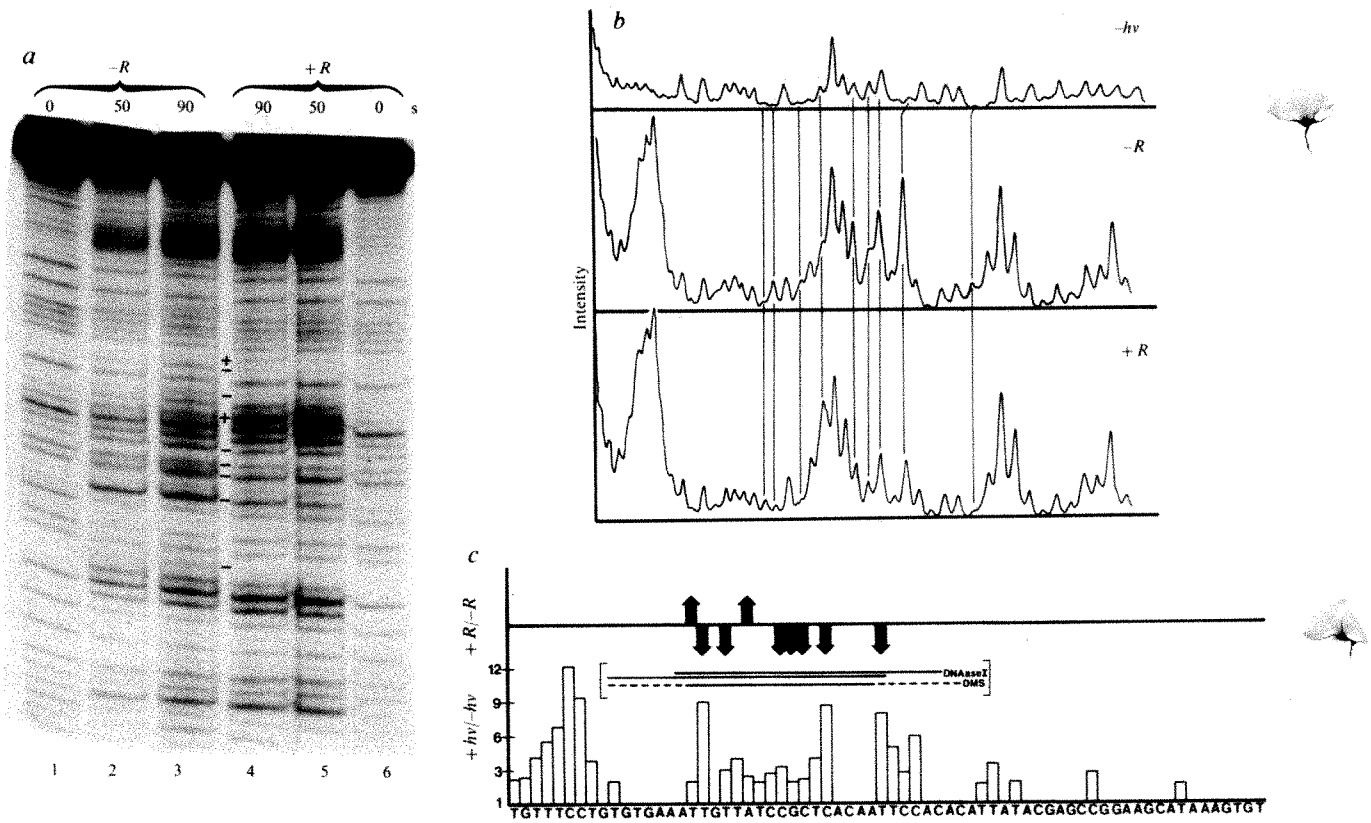


Fig. 3 Photofootprints of the *lac* repressor bound to the *lac* operator *in vivo*. *a*, An autoradiogram of the *lac* repressor photofootprint obtained *in vivo*. Lanes 1–3, the effect of irradiating *E. coli* cells containing the *lac* operator plasmid unbound by *lac* repressor for 0, 50 and 90 s. Lanes 4–6, the effect of irradiating *E. coli* cells containing the *lac* operator plasmid bound by repressor for 90, 50 and 0 s, respectively. *b*, Microdensitometer scans of the 90-s irradiation experiment. The upper portion is the no-light control (lane 6 of *a*; similar results are also observed in lane 1). The middle and lower portions show the effect of irradiating the *lac* operator plasmid in the absence and presence of bound repressor, respectively (lanes 3, 4). *c*, The microdensitometer scans quantified and displayed as a histogram. The effect of light on the cleavage of DNA in the absence of *lac* repressor is expressed as the ratio $(+h\nu/-h\nu)$ while the effect of repressor on light-induced cleavage of DNA is denoted either as an enhancement (↑) or blockage (↓) of cleavage.

Methods: Competent *E. coli* cells lacking a chromosomal copy of the *lac* structural gene (*E. coli* strain E7074 F'lacIProA$^+$, B$^+$Δ(*lac pro*) *sup E thi* (kindly provided by Dr J. Beckwith) were transformed with either plasmid pJW231 or pJW280 (see text for explanation). 900 μl of *E. coli* cells bearing either plasmid were grown to saturation in LB, pelleted and resuspended in 450 μl of M9 buffer[41]. The cells were then placed on ice and photolysed in 150-μl aliquots in a quartz microcuvette. Following irradiation, each aliquot was immediately frozen and stored at −78 °C. The aliquots were combined, pelleted and plasmid DNA isolated by an alkaline-SDS miniprep[42]. After phenol extraction from 1% SDS, the DNA was ethanol precipitated, restricted with *Eco*RI and labelled with [α-^{32}P]dATP using the Klenow fragment of *E. coli* DNA polymerase I. Unreacted ATP was removed by dialysis against 2 M NaCl, 1 mM Tris (*p*H 7), followed by dialysis against 1 mM Tris (*p*H 7). The DNA was then restricted with *Bam*HI, ethanol precipitated and the 95-bp *lac* operator fragment purified by electrophoresis on a 6% polyacrylamide gel. After extraction from the gel and ethanol precipitation, the DNA was processed and analysed as described in Fig. 1 legend.

Although the $NaBH_4$–acidic aniline technique can be used to break the DNA backbone at the sites of photodamage, the efficiency with which the photoproducts are detected is unknown. To estimate this efficiency, a second cleavage technique has been used to labilize the *lac* repressor–*lac* operator photofootprint described in Fig. 1. As shown by others, when UV-irradiated DNA is heated in piperidine the DNA backbone is cleaved quantitatively at the so-called PydC photoproducts[23]. These photoproducts occur at T-C, C-C and to a much lesser extent T-T dinucleotide residues and result from a cyclo-addition reaction between the 5,6-double bond of one pyrimidine and the carbonyl group of thymine or the imino tautomer of cytosine of an adjacent pyrimidine. Because these photoproducts contain a pyrimidine saturated at its 5,6-double bond, their formation can be detected by the $NaBH_4$–acidic aniline cleavage technique outlined here. As Fig. 2 shows, cleavage of the PydC photoproducts by the $NaBH_4$–acidic aniline technique is comparable with the quantitative cleavage of these sites by piperidine (compare methods *b* and *e*). Although these measurements do not indicate the efficiency with which other photoproducts are cleaved by the $NaBH_4$–acidic aniline technique, the structural similarities between the PydC photoproducts and the well known pyrimidine dimers, pyrimidine trimers and spore product suggest that these latter photoproducts[8] will also be readily labilized by this technique.

Photofootprinting the *lac* repressor *in vivo*

Having demonstrated the feasibility of footprinting a protein *in vitro* with light, we next investigated the possibility of footprinting *in vivo* with light. Two derivatives of the plasmid pBR322 have been constructed for this purpose. The first, designated pJW231, contains a copy of the same 95-bp *lac* operator fragment used in the *in vitro* experiments[24]. The second, designated pJW280, contains this *lac* operator plus an 1,100-bp fragment containing the *lac* repressor structural gene under control of an I^q promoter. When *Escherichia coli* cells lacking a chromosomal copy of the *lac* repressor structural gene are transformed separately with these plasmids, two cell types result. *E. coli* cells bearing the pJW231 plasmid contain *lac* operators unbound by *lac* repressor whereas *E. coli* cells bearing the pJW280 plasmid contain *lac* operators bound by *lac* repressor. Following brief irradiation of both cells, plasmid DNA is isolated free of chromosomal DNA. The 95-bp *lac* operator fragment is excised from each plasmid by restriction with *Eco*RI and *Bam*HI, end-labelled with ^{32}P and the photoproducts chemically labilized. The photofootprint of the *lac* repressor is then revealed by denaturing the DNA from both plasmids and comparing their strand breakage pattern on sequencing gels. As shown in Fig. 3, the binding of *lac* repressor *in vivo* enhances the formation of two photoproducts and inhibits the formation of seven others.

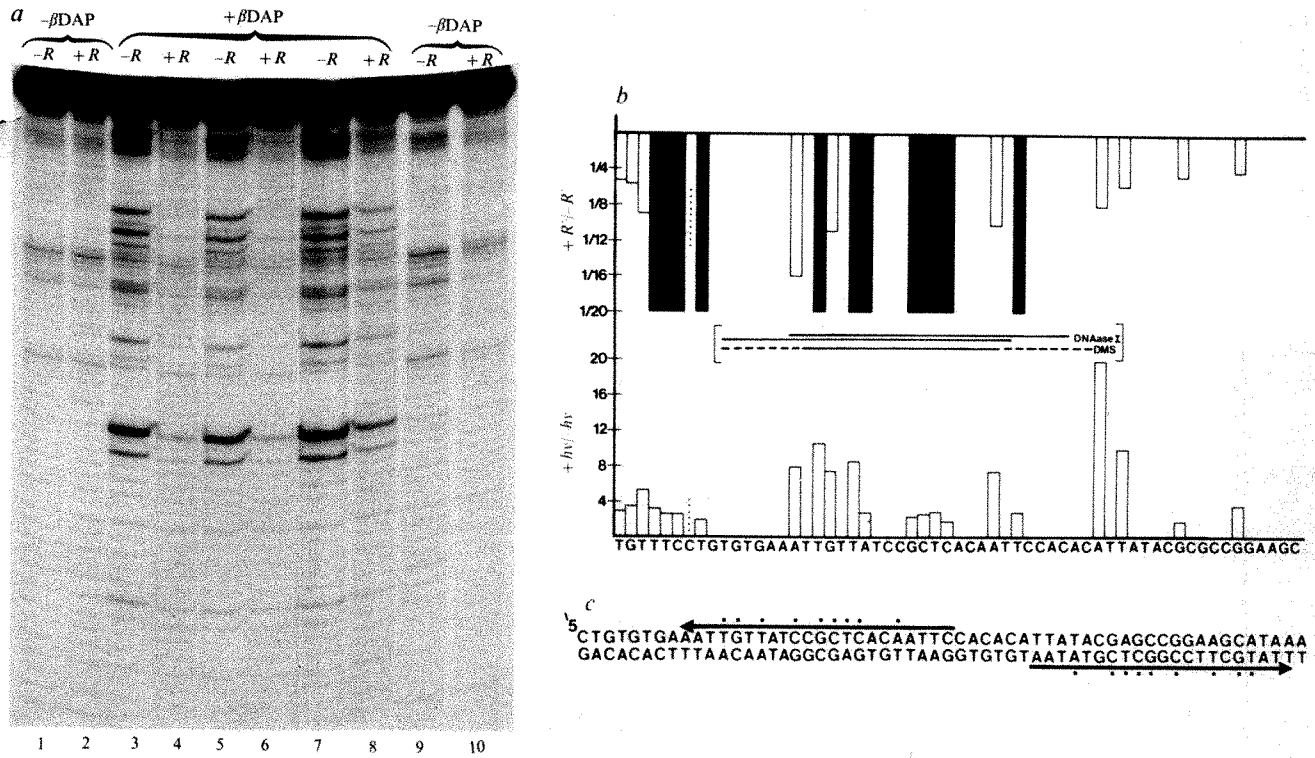


Fig. 4 Photofootprints of the *lac* repressor obtained *in vitro* with a triplet sensitizer. *a*, An autoradiogram of the *lac* repressor–*lac* operator photofootprint. Lanes 1, 2, the effect of a 30-min irradiation of the *lac* operator at 315 nm in the absence of sensitizer. Lane 1 contains no *lac* repressor while lane 2 contains *lac* repressor. Lanes 3–8, the effects of irradiating the *lac* operator with 315 nm light in the presence of 10 mM sensitizer (β-dimethylaminopropiophenone, Aldrich). Irradiation was for 30 min (lanes 3, 4; similar results were obtained in 5 min), 60 min (lanes 5, 6) or 120 min (lanes 7, 8). Odd-numbered lanes contain no *lac* repressor while even-numbered lanes contain *lac* repressor. Lanes 9, 10, the effect of a 120-min irradiation on the *lac* operator at 315 nm in the absence of sensitizer. Lane 9 contains no *lac* repressor while lane 10 contains repressor. *b*, Results of the autoradiogram quantified by microdensitometry and displayed as a histogram. The cleavage of DNA by sensitizer in the absence of *lac* repressor is expressed as the ratio $(+h\nu/-h\nu)$ while the effect of *lac* repressor on sensitizer-induced cleavage is expressed as the ratio $(+R'/-R')$ (see Fig. 1 for nomenclature). A base whose cleavage could not be accurately quantified by microdensitometry is denoted by a dotted line. Fading of the photofootprint at long irradiation times is probably due to damage of the complex by the 315 nm light. *c*, The plausible pseudooperator site. Homologous bases are denoted by a dot.
Methods: Irradiation was with the same apparatus as described for Fig. 1 except that a monochromator (Instruments SA, Inc., Model 1200-UV-H10) was placed between the water filter and the sample. The monochromator entrance and exit sites were 2 mm (15 nm bandwidth). The experimental conditions and reagents were the same as for Fig. 1.

The *lac* repressor binding site detected in these measurements agrees well with the *in vitro* photofootprinting results as well as with the *in vitro* results obtained by other techniques[19–21]. Thus, contacts between the *lac* repressor and its operator can readily be detected *in vivo* with light.

A triplet sensitizer and psoralen

Although light alone can be used to detect *lac* repressor and, as shown below, RNA polymerase binding, less tightly associated proteins might go undetected. We have therefore sought to develop additional photochemical techniques which may be more sensitive to these types of protein–DNA interactions. Two such techniques have been developed. In the first, a triplet sensitizer, β-dimethylaminopropiophenone (βDAP) is used to excite DNA indirectly. As demonstrated previously, when excited by 315 nm light *in vivo*, βDAP can transfer its excitation energy to DNA, causing the DNA to assume an excited triplet state[25]. Because this triplet state yields many of the same photoproducts formed by direct UV irradiation of DNA, the chemical labilization procedure outlined here can be used to detect βDAP-induced photolesions. The major advantage of using a sensitizer to introduce photolesions into DNA is the requirement of a collision between sensitizer and DNA before the sensitizer can transfer its excitation energy to DNA[26]. Thus, photoproduct formation induced by βDAP is expected to be more sensitive to protein binding than direct irradiation by UV light.

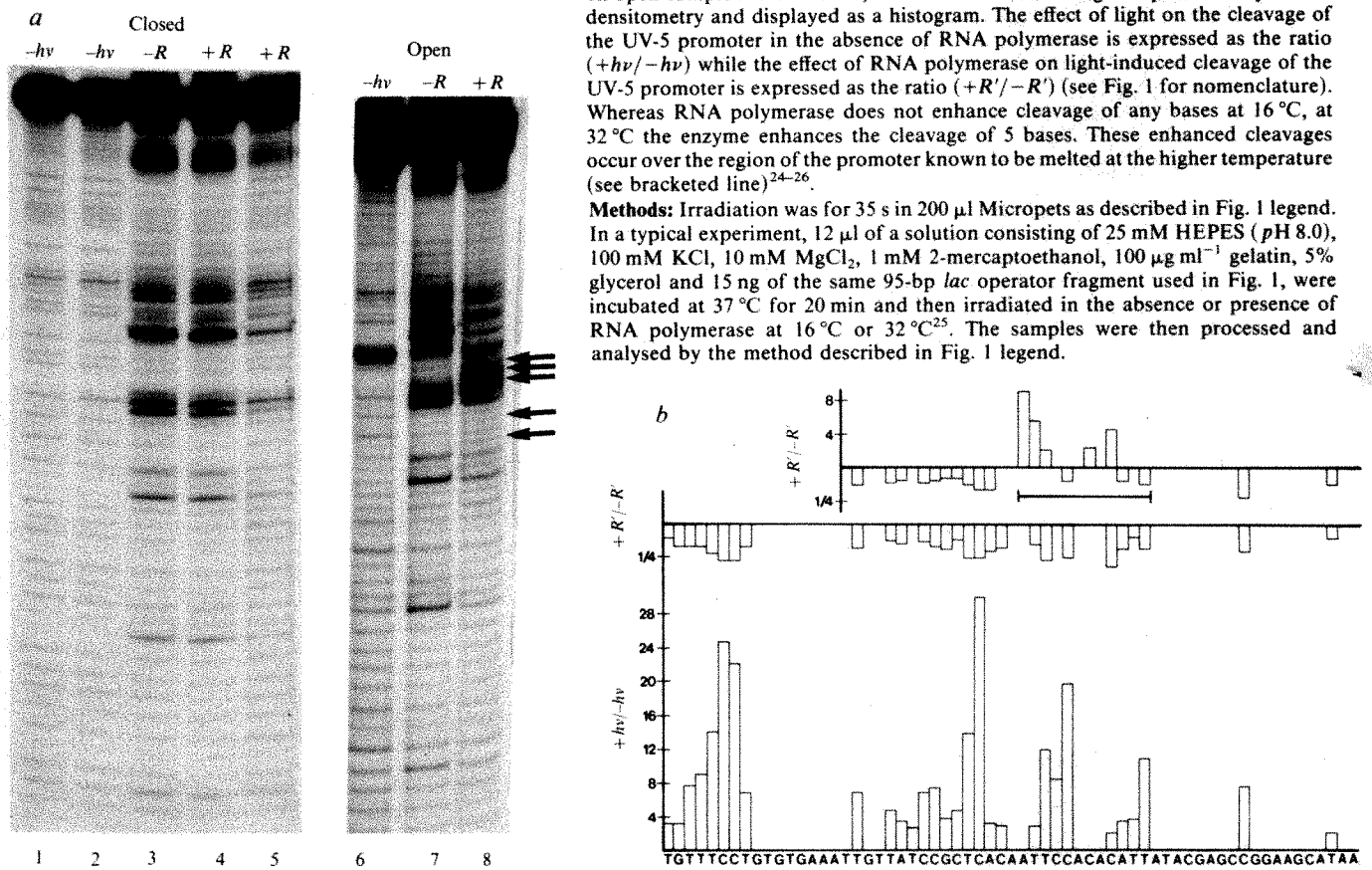
To test these hypotheses, the same *lac* repressor–*lac* operator experiment described in Fig. 1 was repeated using 315 nm light to excite the sensitizer. As shown in Fig. 4, in the absence of sensitizer, DNA is undamaged by 315 nm radiation, but in the presence of sensitizer, irradiation introduces a larger number of photoproducts into the DNA. When these experiments are repeated in the presence of *lac* repressor, repressor binding strongly inhibits the formation of many of the βDAP-sensitized photoreactions.

The *lac* repressor photofootprint obtained with βDAP exhibits many of the same features observed by other techniques. For example, that region of the operator strongly footprinted by bromouracil cross-linking[4], DNase I digestion[20] and dimethyl sulphate (DMS)[19] is also strongly protected from βDAP-induced cleavage. However, in contrast to these measurements, βDAP also detects *lac* repressor contacts outside the presumed operator. Strong contacts are observed downstream from the operator while weaker ones are detected upstream. Although strong contacts downstream are not detected by bromouracil cross-linking or DMS experiments, reexamination of the DNase I results[20] reveals that *lac* repressor both enhances and inhibits DNase I cleavages in this region. As photofootprinting with UV light also detects repressor contacts in this region (see Fig. 1), strong inhibition of sensitizer-induced cleavage in this area probably results from repressor contacts that are insensitive to bromouracil cross-linking or DMS protection experiments.

Weak contacts detected upstream from the presumed *lac* operator with βDAP are also detected by direct irradiation with UV light (see Fig. 1). Because some of these cleavages occur well upstream of the presumed operator, we have examined the sequence of this area for the possibility of a second or pseudo-operator binding site. As shown in Fig. 4, we indeed found a

Fig. 5 *In vivo* photofootprints of *E. coli* RNA polymerase bound to the *lac* UV-5 promoter in a closed and open complex. *a*, An autoradiogram of the RNA polymerase photofootprint. Lanes 1–5, photofootprints of the closed RNA polymerase complex; lanes 6–8, photofootprints of the open RNA polymerase complex. Lanes 1 and 2 are no light controls. Lane 1 contains no RNA polymerase whereas lane 2 contains 100 nM RNA polymerase. Lane 3 shows the effect of irradiating the UV-5 promoter at 16 °C in the absence of RNA polymerase. Lanes 4 and 5 show the effect of irradiating the UV-5 promoter at 16 °C in the presence of 50 and 100 nM RNA polymerase, respectively. Lane 6 is a no-light control for the open complex. The dark band approximately one-third of the way down from the top of the gel is a contaminating *Eco*RI* band present in this preparation of DNA. It results from overdigesting the plasmid pJW231 with *Eco*RI and *Bam*HI to liberate the desired 95-bp UV-5 promoter fragment used in these photofootprinting experiments. Lane 7 shows the effect of irradiating the UV-5 promoter at 32 °C in the absence of RNA polymerase. Lane 8 shows the effect of irradiating the UV-5 promoter at 32 °C in the presence of 100 nM RNA polymerase. Arrows indicate the 5 bases showing the enhanced photoreactivity on open complex formation. *b*, Results of the autoradiogram quantified by microdensitometry and displayed as a histogram. The effect of light on the cleavage of the UV-5 promoter in the absence of RNA polymerase is expressed as the ratio $(+h\nu/-h\nu)$ while the effect of RNA polymerase on light-induced cleavage of the UV-5 promoter is expressed as the ratio $(+R'/-R')$ (see Fig. 1 for nomenclature). Whereas RNA polymerase does not enhance cleavage of any bases at 16 °C, at 32 °C the enzyme enhances the cleavage of 5 bases. These enhanced cleavages occur over the region of the promoter known to be melted at the higher temperature (see bracketed line)[24–26].

Methods: Irradiation was for 35 s in 200 μl Micropets as described in Fig. 1 legend. In a typical experiment, 12 μl of a solution consisting of 25 mM HEPES (*p*H 8.0), 100 mM KCl, 10 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 100 μg ml^{-1} gelatin, 5% glycerol and 15 ng of the same 95-bp *lac* operator fragment used in Fig. 1, were incubated at 37 °C for 20 min and then irradiated in the absence or presence of RNA polymerase at 16 °C or 32 °C[25]. The samples were then processed and analysed by the method described in Fig. 1 legend.



second potential operator sequence, identical with the known *lac* operator at 9 bases. The observed 9-bp homology makes this site a reasonable candidate for repressor binding because a known repressor binding site, just upstream from the 'cap' binding site of the *lac* operon, also exhibits a 9-bp homology with the *lac* operator[27]. Binding studies of the *lac* repressor to a restriction fragment bearing both of these 9-bp operator sequences plus the known *lac* operator shows the formation of three strong complexes with relative affinities of 1, 1/200 and 1/800 (ref. 28). Although these measurements did not locate the weakest binding site, the affinity observed for this site is compatible with binding at the proposed site in Fig. 4, as the other 9-bp operator binding site exhibits only a fourfold greater affinity.

Although repressor binding at the proposed pseudo-operator sequence in Fig. 4 was not reported in DNase I protection experiments[20], reexamination of these results does reveal weak protection in this area. The failure of bromouracil cross-linking and DMS protection experiments to detect this repressor binding site may be due to the fact that a restriction fragment bearing only one-half of this operator was used in those experiments.

A second small organic molecule examined for its potential to footprint DNA photochemically is the well known intercalator psoralen. When excited by 350 nm radiation, psoralen covalently binds to DNA[29,30]. Because covalent addition saturates the 5,6-double bond of pyrimidines[31], the chemical labilization procedures used to detect UV and sensitizer-induced photoproducts can also be used to detect psoralen binding. A major advantage of using psoralen is its known sensitivity to nucleosome binding. Previous studies have shown that *in vivo* psoralen does not photoreact with nucleosome-bound DNA.[32,33] We have determined that psoralen binding can be detected at the base pair level using the chemical degradation procedure and that such binding is sensitive to *lac* repressor binding. Experiments are being conducted to determine whether nucleosome binding can be detected with psoralen, UV light and βDAP.

Photofootprinting structural changes in DNA

Previous measurements have shown that UV-induced damage to DNA may be enhanced by structural changes in the DNA. For example, pyrimidine dimer formation is enhanced in single- versus double-stranded DNA[8]; apparently, geometrical changes necessary for dimer formation are easier in single-stranded DNA. We have examined the separation of DNA strands by RNA polymerase to establish whether light can be used to footprint structural changes in DNA.

At low temperatures, *E. coli* RNA polymerase forms a closed complex at promotors[23]. When the temperature of this complex is raised, a short stretch of DNA is unpaired by RNA polymerase, resulting in the formation of a transcriptionally active open complex[34–37]. Photofootprinting of both the open and closed complexes of RNA polymerase bound to the *lac* UV-5 promotor revealed that the formation of both complexes can readily be detected with UV light (Fig. 5), binding being characterized by reductions in photoproduct formation throughout the promoter. However, whereas the closed complex does not enhance the photoreactivity of any bases, the open complex

enhances the formation of five photoproducts. The location of these five enhanced photolesions is in excellent agreement with the known site of DNA strand separation determined by other measurements[35–37]. Thus, a small structural change in DNA, such as its unpairing by RNA polymerase, can readily be detected at the base pair level by light.

Conclusions

The photochemical approaches used in these experiments to footprint DNA have several advantages over other methods. First, since light readily permeates cells, DNA can be footprinted *in vivo*. Indeed, when combined with the procedure of sequencing genomic DNA directly by indirect end-labelling[38], it should be possible to footprint almost any gene *in vivo* with light. Second, because the footprinting reaction is directly coupled to the absorption of light, it can be initiated essentially instantaneously and terminated within 10^{-9} to 10^{-6} s (the lifetime of the excited state). Thus, by using more powerful light sources it should be possible to footprint, kinetically, structural changes in DNA as well as protein movement along the DNA. Third, because the quantum yield of a photochemical reaction is relatively insensitive to changes in temperature or solvent, footprints of DNA can be obtained in a wide range of conditions. Fourth, because the chemical procedure developed to labilize DNA photoproducts is also expected to work on RNA, it should be possible to photofootprint RNA as well as DNA.

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LETTERS TO NATURE

Search for excess cosmic-ray events from the galactic plane

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Wdowczyk and Wolfendale[1] have recently suggested that the cosmic-ray beam at 10^{15}–10^{16} eV may contain a significant flux of γ rays from the direction of the galactic plane, and that this flux may affect the measured all-particle anisotropy and energy spectrum at these energies. We have searched for an excess of cosmic-ray events from the direction of the galactic plane. We can set upper limits which are close to the flux predicted by Wdowczyk and Wolfendale for the full galactic plane, and also for regions around the galactic centre. We have used these upper limits to place limits on the apparent brightness distribution of galactic γ-ray sources above 10^{15} eV, and on any contribution these sources can make to the cosmic-ray energy spectrum at the highest energies.

Recent observations using extensive air shower techniques have provided evidence for galactic sources[2–6], and possibly extragalactic sources[7], capable of producing γ rays with ultra high energies (10^{15}–10^{16} eV). Wdowczyk and Wolfendale[1] have examined the possibility that such galactic γ rays might be responsible for structure found in the cosmic-ray energy spectrum and anisotropy at these energies, and concluded that there is some experimental evidence for this. No individual datum is conclusive but the cited evidence suggests that γ rays may be responsible for 1% of cosmic ray showers from the direction of the galactic plane ($|b| < 10°$), and a rather larger fraction from near the galactic centre. This would constitute ~0.2% of the primary cosmic radiation.

We have used data from 3yr of operation of the University of Adelaide extensive air shower array at Buckland Park[8] (1.3×10^5 events with a mean energy of $\sim 5 \times 10^{15}$ eV) to search for any excess from the galactic plane. We summed all events observed with galactic latitude ($|b|$) below ~10° and compared this sum with that expected on the basis of events observed outside the galactic plane but at similar declinations. The data from Buckland Park are presented in Table 1 together with data from three other experiments[9–11] operating in a similar energy range. This then gives a full, though non-uniform, coverage of the galactic plane. The data from the experiments by Clark[9] and Chitnis *et al.*[10] were extracted from published celestial intensity maps. The data from Haverah Park were obtained with their 50-m system and were extracted from ref. 11. In the latter cases, the original investigators have presented their own null results for $|b| < 20°$ (ref. 9), $|b| < 10°$ (ref. 10) and $|b| < 5°$ (refs 11, 12). The Haverah Park and MIT experiments observed essentially the same region of sky. Data from these two experiments have been combined and are also shown in Table 1, clearly there is no evidence for there being any enhancement for $|b| \leqslant 10°$.

In estimating any upper limits to a γ-ray contribution from the galactic plane, one must take into account the statistical uncertainty in the cosmic-ray background estimation[13]. This depends on the total number of events used to estimate the background. The ratio, α, of the estimated background counts for $|b| \leqslant 10°$ to the total number of events with $|b| \geqslant 10°$ which were used to estimate the background is given in Table 1 for each experiment. Recently, Protheroe[14] has derived 95% (~1.65 s.d.) upper limits to the ratio of the source contribution to the background contribution given various observed counts,

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Table 1 Observed and expected number of events from $|b| \leqslant 10°$ for various ranges of galactic longitude

Experiment	Galactic plane					Galactic centre region	
	Buckland Park	Kodaikanal	MIT	Haverah Park	Haverah Park + MIT	Buckland Park	Buckland Park
Lat.	35° S	10° N	42.5° N	54° N	—		
Ref.	This work	10	9	11	9, 11		
Approximate range of galactic long.	210°–30°	0°–80° and 170°–240°	40°–200°	40°–200°	40°–200°	350°–10°	300°–60°
No. observed	21156	13,555	552	99,408	99,960	4,631	10,221
Estimated background	21103	13,529	530	99,421	99,951	4,726	10,258
α	0.191	0.184	0.251	0.276	0.276	0.0833	0.0846
γ-ray/cosmic ray upper limits (%)							
84%	1.2	1.5	10.0	0.50	0.51	1.3	1.3
95%	1.7	2.0	13.0	0.70	0.71	2.0	1.8

84% and 95% confidence upper limits are given for the ratio of γ-ray flux to cosmic-ray flux.

estimated background, and α values. The procedure involved estimating the joint probability density function of the true background having some value and the ratio of source contribution and background having some value, and is described fully in ref. 14. The 95% confidence upper limit to the ratio of γ-ray contribution to cosmic-ray contribution for $|b| \leqslant 10°$ has been calculated for each experiment using this procedure and is given in Table 1 which also shows, 84% (~1 s.d.) upper limits calculated in the same way. Only the Haverah Park data have a 95% confidence upper limit to a galactic plane γ-ray contribution which is below ~1% of the cosmic-ray contribution.

By taking the lowest upper limit from the experiments which cover a particular region of the galactic plane and integrating over galactic longitude, we can obtain an estimate of the upper limit to any γ-ray enhancement for the galactic plane as a whole. Doing this, we obtain an 84% confidence upper limit of ~0.9%, and a 95% confidence upper limit of ~1.3%, for any γ-ray enhancement from the whole galactic plane ($|b| \leqslant 10°$). These upper limits are close to the ~1% enhancement predicted by Wdowczyk and Wolfendale[1].

The galactic centre passes almost overhead at Adelaide. We have used our data to search for any enhancement from the galactic plane within 60° of the galactic centre. It is possible that the galactic centre has a more localized excess and we have also examined events from the direction of the galactic centre (within ~10°). The results are also given in Table 1 together with the upper limits we derive for any γ-ray enhancement from these regions of the sky. The data show no significant enhancement over the expected background. Again, however, the 95% upper limits are very close to the flux predicted by Wdowczyk and Wolfendale[1].

At 10^{15}–10^{16} eV, the overall cosmic-ray anisotropy has a first harmonic amplitude of ~0.3% (ref. 1). Our limits on any excess flux from the galactic plane appear to preclude the possibility that such a high amplitude for a whole sky anisotropy could result solely from galactic γ rays.

There is no doubt that an excess of γ-ray events should exist from the galactic plane due to known galactic ultra high energy γ-ray sources. None of these sources has, however, been associated with large numbers of γ rays arriving at the Earth. The γ-ray sources which have been observed each contribute ~0.1% of the total cosmic ray events detected from the galactic plane by each experiment. Our 95% confidence upper limit of 1.3% for the total γ-ray excess from the galactic plane thus corresponds to only ~40 times the observed flux from a single known source.

Cygnus X-3 (refs 2, 3) is one of the brightest and also the most distant of the known galactic sources, being at a distance of the order of the galactic radius. This suggests that a simple disk population brightness, S, distribution (that is $N(>S) \propto S^{-1}$) is unlikely to be valid, and that instead the distribution of observed fluxes reflects the (unknown) γ-ray luminosity function of the sources.

Cygnus X-3 is not in the direction of the galactic centre. Neither are the Crab pulsar[5,6] and Vela X-1 (ref. 4), the other galactic ultra high energy candidate sources. Our upper limits on the inner galaxy flux ($|b| < 10°$, $|l| < 60°$) will not allow an enhancement above the total flux of outer galaxy sources such as is found for X rays $(9.4\times)$[15] or γ rays above 100 MeV $(2.2\times)$[15] even if these three sources were the only sources contributing to the 10^{15}–10^{16} eV γ-ray flux from the outer galaxy.

We note that if the total flux of γ rays were lower by about 10 times than that postulated by Wdowczyk and Wolfendale (it cannot be much lower than this on the basis of known sources) and their spectrum extends to $\geqslant 10^{19}$ eV, these events would distort the observed cosmic ray spectrum and produce a flattening at $\sim 10^{18}$ eV. With some steepening in the γ-ray spectrum above 10^{16} eV they would instead produce an 'ankle' in the cosmic ray spectrum as observed[16] at 10^{19} eV. It may then not be necessary to postulate a sharp cutoff to the γ-ray spectrum at 10^{17}–10^{18} eV which Wdowczyk and Wolfendale[1] needed to avoid gross disagreement with the observed cosmic-ray spectrum. Support for this suggestion may be found in data for energies above 10^{19} eV presented by the Sydney group[17]. These data show an excess from the galactic plane for showers with energies above 4×10^{19} eV (six events are observed for $|b| < 10°$ where we expect 1.0 ± 0.5). In this case, the Sydney detectors would be predominantly detecting muons from photoproduced pions[18–19]. At these energies the flux of such muons is expected to be quite significant for γ-ray initiated showers.

We have set a 95% confidence upper limit of 1.3% for any enhancement by γ rays of the 10^{15}–10^{16} eV cosmic ray flux from the galactic plane. This sets a limit to the total flux of ultra high energy galactic γ rays at ~40 times that observed from Cygnus X-3. Cygnus X-3 must then be amongst the brightest sources of such γ rays in our galaxy. These γ rays may be responsible for the ankle in the cosmic-ray energy spectrum above 10^{19} eV.

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Radio wave scattering in the galactic disk

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Electron density turbulence in the Galaxy causes radio sources to be broadened angularly by amounts that depend strongly on the direction of the source. Recent scattering measurements using very long baseline interferometry (VLBI)[1] and interplanetary scintillations (IPS)[2] of extragalactic sources, and interstellar scintillations (ISS) of pulsars[3] are used here to constrain the distribution of scattering material in the Galaxy. Although ubiquitous, scattering material appears to be highly concentrated in a thin disk of ~100 pc thickness and in clumps of 1–10 pc size probably associated with H II regions, stellar wind bubbles, and/or supernova shocks. Typical transverse velocities of scattering material are much smaller than typical pulsar velocities. We predict the scattering angle as a function of galactic latitude. The results imply a maximum observable surface brightness for incoherent sources of electron synchrotron radiation.

Recent work has demonstrated that electron density fluctuations on length scales of 10^9–10^{11} cm conform to a power law wavenumber spectrum of the form $C_n^2 q^{-\alpha}$ with $\alpha = 3.6 \pm 0.2$ (refs 3–6) where q is the wavenumber. This form of the spectrum is consistent with detailed measurements on a few lines-of-sight. Less detailed measurements on others sometimes suggest different values of α (refs 3, 7). We adopt a specific value ($\alpha = 11/3$) in the analysis below which is in agreement with the best studied lines-of-sight. Variations of α with direction will not grossly modify any of our calculations. There is some evidence that the spectrum extends over six or more decades in wavenumber, q (ref. 6). Although the slope of the spectrum shows little variation, the level of turbulence C_n^2 varies by at least four decades, both in going from high to low galactic latitudes and between lines-of-sight confined to the galactic disk[3].

We now use scattering and scintillation observables to constrain further the distribution of scattering material in the Galaxy and to provide a model for the observed scattering disk size as a function of galactic coordinates. VLBI measurements determine the scattering angle θ_{ISS} (FWHM) directly. IPS yields angular sizes by comparing the scintillation power spectrum of a source seen through the interplanetary medium with that of a point calibrating source at the same solar elongation. Interstellar scintillations of pulsars are parameterized by the scintillation bandwidth Δf_{ISS} and scintillation fluctuation time Δt_{ISS} (both half widths at half maxima of the two-dimensional intensity correlation function). The scattering angle for a plane-wave source is[3,8] (with C_n^2 in units of $m^{-20/3}$)

$$\theta_{ISS} \approx 0.133 \, (L_{kpc} \langle C_n^2 \rangle)^{3/5} f_{GHz}^{-11/5} \quad \text{arc s} \qquad (1)$$

where L is the effective path length in kpc, f is the observation frequency in GHz, and $\langle\ \rangle$ denotes average over path length. The wavenumber that contributes most to the scattering of a given source (if all turbulence were confined to a thin layer at distance $L/2$) is $q_{eff} \approx 2\pi\theta_{ISS}/\lambda$. Relative motion of source, turbulence and observer causes time variations with an effective velocity.

$$V_{ISS} \approx 10^{4.4} (L_{kpc} \Delta f_{ISS})^{1/2} / \Delta t_{ISS} f_{GHz} \quad \text{km s}^{-1} \qquad (2)$$

(Δf_{ISS} in MHz) which has been shown to correlate at the 66% level with pulsar velocities obtained interferometrically[9]. Consequently it appears that V_{ISS} is dominated by the pulsar velocity rather than that of the scattering medium. This would seem to rule out enhanced ISS from stellar winds themselves which, by analogy with IPS and the solar wind, would yield $V_{ISS} \sim 10^2$–10^3 km s^{-1}.

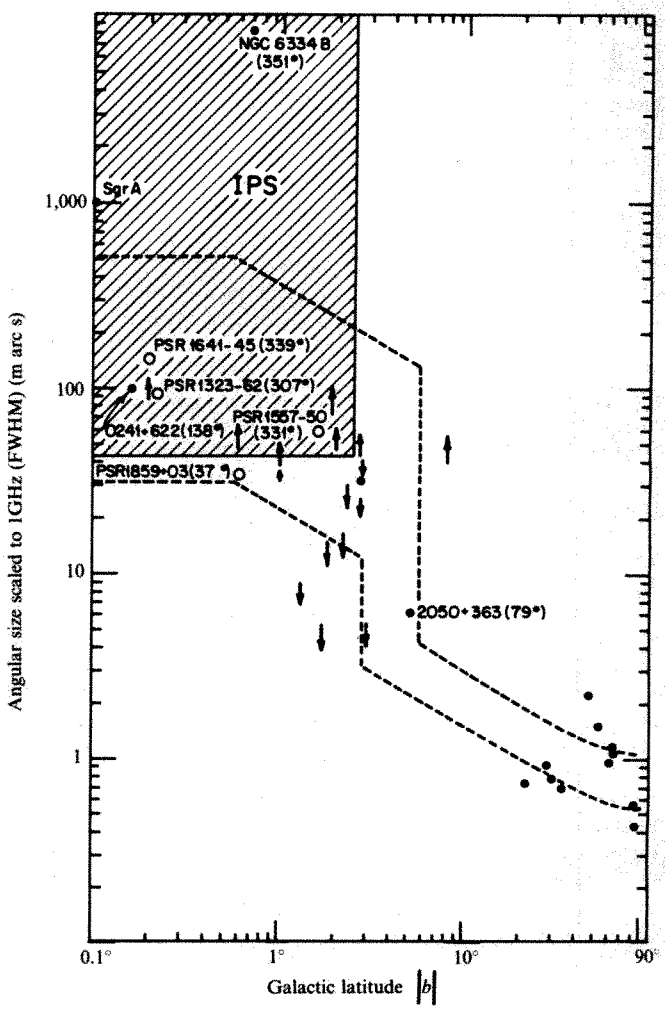


Fig. 1 Scattering diameter at 1 GHz versus galactic latitude for the inner galaxy. ●, VLBI measurements of extragalactic sources[1,26,27] and arrows represent lower and upper limits. The point for NGC6334B is an extragalactic source viewed through the H II complex NGC6334 (ref. 28). ○, Implied angles derived from pulsar scintillation measurements and the effective scattering angle for a source of plane waves at the distance of the pulsar (5–10 kpc). The shaded region summarizes the IPS observations of sources in the direction of the galactic centre[2] and the dashed lines are predicted angles from a survey of pulsar ISS[3].

Pulsar ISS measurements for 76 objects suggest a two-component model for C_n^2: component A with scale height $H_A \geq 0.5$ kpc, $C_n^2 \sim 10^{-3.5}$, and volume filling factor ~1; component B wherein C_n^2 is large in clump-like regions of size $R_{clump} \sim$ 1–10 pc with very small filling factor $\sim 10^{-4}$–10^{-3}, and small scale height, $H_B \sim 50$–100 pc (ref. 3). The pathlength-averaged C_n^2, $\langle C_n^2 \rangle$, increases systematically with decreasing galactocentric radius, suggestive of component B clumps being more common at galactocentric radii smaller than that of the Sun. However, the increase may also reflect an increasing probability for encountering clumps with increasingly larger internal C_n^2. The anticentre directions yield $\langle C_n^2 \rangle$ that are typically smaller than those within the solar circle for the same distance, so that there seems to be a real galactocentric radial dependence. The mean free path for encountering clumps is $L_{mfp} \sim 2$–10 kpc so lines of sight with $|b| \geq 10°$ sample only the 'A' component.

The pulsar results are in good agreement with both recent VLBI and IPS measurements. A recent survey[1] of low-latitude ($|b| < 5°$) compact extragalactic radio sources at 408 MHz shows that 8 out of 18 sources towards the inner Galaxy ($10° \leq l < 80°$)

are sufficiently scattered to yield no fringes implying, typically, $\theta \geq 0.2$–0.3 arc s. By comparison, all 11 sources within $197° < l < 234°$ had $\theta < 0.2$ arc s and only one showed no fringes, probably because it lies behind an H II region. The VLBI results are consistent with a 50% probability for heavy scattering of extragalactic sources in directions $10° \leq l \leq 80°$ and $|b| \leq 1°$. A search for sources that display IPS[2] at 327 MHz in the regions $-10° < l < 30°$ and $180° < l < 210°$ with $|b| < 12°$ show a marked lack of scintillating sources towards the inner Galaxy. Only two out of an expected eight such sources were detected above a flux density of 0.2 Jy for $|b| < 2.5°$, suggesting that the scattering disk due to interstellar scattering ≥ 0.5 arc s, typically.

We combine all data for the inner galaxy in Fig. 1, which shows the equivalent scattering angle (FWHM) at 1 GHz after scaling all measurements according to $\lambda^{2.2}$. VLBI measurements, upper limits, and lower limits are shown as dots or arrows, the IPS results are summarized as a shaded region which designates $\theta > 43$ m arc s for $|b| < 2.5°$, and the pulsar measurements are displayed as predictions for the scattering angle derived from the two-component model. We also show the implied scattering angles for four highly scattered pulsars. Two prediction curves are shown which bracket the expected range of angles. For $|b| \geq 3°$–$6°$, scattering angles are determined by the 'A' medium for nearly all lines-of-sight and are deterministic. For smaller latitudes the scattering angle is a random variable (associated with the probability of intersecting a component B clump) and may fall outside the predicted range, as some of the upper limits apparently do. Break points in the prediction curves occur at angles $\sim H_B/L_{max}$ and $H_A/L_{max} \sim H_B/L_{mfp}$ where L_{max} is the maximum possible pathlength, taken to be 10 kpc. Apart from the break points and the constant parts of the curves for $|b| \leq 0.7°$, the scattering angle varies as $\sim |\sin b|^{-3/5}$ but with different proportionality constants for different ranges of b. We note that our results for $|b| \geq 10°$ agree with previous results based solely on IPS measurements[10,11].

The predicted scattering angle can be considered the apparent diameter of a scattered point source only as long as the condition of strong scattering holds[8]. Roughly, this is true if $q_{eff} \geq q_{Fresnel} = 2\pi/\sqrt{\lambda L}$ or $\theta_{ISS} \geq \sqrt{\lambda/L}$. At smaller λ, the scattered source brightness distribution takes on a core–halo form (for intrinsic source size $\ll$ scattering disk size). At $b = 90°$, $\theta_{ISS} \sim 1$ m arc s at 1 GHz implies that strong scattering occurs for $\lambda \geq \lambda_c \sim 4$ cm. At directions towards the inner Galaxy with $|b| \leq 1°$, $\lambda_c \sim 0.12$ cm for $\theta_{ISS} \sim 100$ m arc s and an effective screen distance of 5 kpc. The compact source Sgr A at the galactic centre with $\theta_{ISS} \sim 1$ arc s at 1 GHz (ref. 12) yields $\lambda_c \sim 0.3$ mm if the apparent source size at decimetric wavelengths is indeed due to scattering. Although free–free absorption[13] from gas with a density $\propto r^{-1}$ can also produce a wavelength dependence $\theta \propto \lambda^2$ and the angular size is an order of magnitude larger than the most scattered pulsar (PSR1641 − 45), the necessary amount of scattering (from the general galactic turbulence as opposed to turbulence local to the source) is not an unrealistic extrapolation of the pulsar results. This follows because Sgr A is further than the most scattered pulsar and the line-of-sight passes through much more ionized material. The pulsar results themselves indicate that θ_{ISS} increases much more rapidly than the $L^{3/5}$ variation that would be expected for a medium with a uniform level of turbulence. We also note that scattering may produce asymmetric images such as has been reported for Sgr A[14]. Turbulence in an anisotropic medium, that is, one with a preferred direction associated with a velocity flow, magnetic field or gradient in the level of turbulence, has an anisotropic wavenumber spectrum which will yield an asymmetric scattering disk. As yet there is no evidence for such an asymmetry from observations of astronomical sources. IPS measurements at 74 MHz (ref. 15) show asymmetric diffraction patterns which are thought to derive from the intrinsic source structure of extragalactic sources because the Crab pulsar, after angular broadening due to ISS, shows no asymmetries in its IPS diffraction. The possibility still remains, however, that sources showing large amounts of scattering will be broadened asymmetrically.

The available evidence implies that scattering occurs along every line-of-sight, albeit by vastly different amounts. Turbulence extends to at least 0.5 kpc in a diffuse component but has large amplitudes in clumps whose distribution resembles a population I class of objects. Pulsars that are known to have intervening H II regions (PSR0611 + 22) or supernova remnants (Crab and Vela) all show enhanced scattering. However, there is no knowledge of the relative importance of a large mean electron density and the energization required to drive the turbulence. What analysis exists implies that considerable energy is required to maintain the density fluctuations[16].

Because of its ubiquity, ISS has important consequences concerning measurements of intrinsic source sizes, ψ. In the conditions $\theta_{ISS} \geq \psi \geq \zeta\theta_c$ where $\zeta \geq 1$ is a dimensionless numerical factor and $\theta_c \sim \lambda/2\pi L\theta_{ISS}$ is a critical angular size, the brightness distribution is irretrievably broadened into a distribution of width θ_{ISS}[17,18]. Only for $\psi \leq \theta_c$ is the radiation field coherent over the scattering disk, a requirement for observing fully modulated interstellar scintillations and for determining ψ from interferometric observations, provided the bandwidth and integration time are less than those characterizing the ISS (for example, Δf_{ISS} and Δt_{ISS} defined above)[17,18]. ISS observations can probe intrinsic sizes with $\zeta = \psi/\theta_c \leq 10^3$ by measuring intensity variations down to a modulation index $m_{ISS} \sim \zeta^{-1} \sim 0.1\%$ (smaller indices are probably impossible to measure because of instrumental effects[19–23] and therefore $\psi \gg \theta_c$ for these sources). (Recent work[24] has suggested that intensity variations of extragalactic sources may be caused by refraction from galactic electron density turbulence. This possibility is peripheral to the issues we discuss here.) Although new classes of extragalactic objects may be discovered which satisfy $\psi \leq \theta_c$, at present it seems that $\psi \gg \theta_c$ for extragalactic sources and therefore any interferometer observation will measure an angular size θ_{ISS} if $\psi < \theta_{ISS}$ and $\lambda > \lambda_c \sim 4$ cm (observing perpendicular to the galactic plane). Therefore, interferometer observations with baselines $\sim 10 R_{Earth} f_{GHz}^{1.2}$ will tend to be dominated by ISS, and baselines twice this size will totally resolve the scattering disk (for $f \leq 7$ GHz or $\lambda > \lambda_c \sim 4$ cm). Taking $\theta_{ISS} = 0.5$–2.0 m arc s at 1 GHz, we find that the maximum observable brightness temperature for high-latitude sources is

$$T_{b\,max} \approx 10^{12.1 \pm 0.6} S_{Jy} f_{GHz}^{2.4}\ \text{K}, f \leq 7\ \text{GHz} \qquad (3)$$

Therefore, intrinsic source structure having a brightness temperature close to or in excess of the inverse Compton limit ($\sim 10^{12}$ K) will be difficult, if not impossible, to resolve at frequencies < 1 GHz. We point out, however, that the detection of diffraction-produced intensity variations at sub-GHz frequencies would necessarily imply $T_b > 10^{12}$ K (refs 19–21).

The only known class of sources for which these limitations can be overcome are pulsars, which show interstellar scintillations and whose emission regions satisfy $\psi < \theta_c$ (ref. 25). VLBI observations with bandwidth and integration time smaller than Δf_{ISS} and Δt_{ISS}, respectively, will yield fringes on baselines $\leq \lambda/\psi$ whose phase and amplitude vary randomly on a time scale Δt_{ISS}. Consequently, fringes should be detectable out to at least 1 AU for a typical pulsar at 1 kpc distance and probably on much longer baselines because emission regions are evidently much smaller than the light cylinder radius[25].

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Hercules X-1—a 1,000 GeV γ-ray pulsar

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An X-ray pulsar has been detected for the first time as a very high-energy (VHE) γ-ray pulsar. X-ray emission from Hercules X-1 is multiply periodic with a pulsar period of 1.24 s, a binary orbital period of 1.7 day and a 35-day amplitude modulation of unknown origin characterized by a sharp turn-on followed by a slow decline over 11 days. During a drift-scan we have detected a significant 3-min outburst of VHE γ rays, which had a 1.24-s periodicity. This outburst occurred 35 days before an observed X-ray turn-on. The γ-ray luminosity during the outburst was of the same order as the X-ray luminosity. Together with the coincidence in time, this suggests a connection between the mechanisms giving rise to the γ-ray outburst and the X-ray turn-on. Subsequent monitoring of the source during a period when the X-ray flux was decreasing yielded some evidence of much weaker pulsed γ-ray activity at the pulsar period. We note a possible similarity between this system and Cygnus X-3.

The discovery of Her X-1 as a pulsing (1.24-s period), eclipsing binary X-ray source was made by the UHURU group[1]. The optical counterpart was identified as Hz Herculis[2] following an improved positional determination for the X-ray source and the detection of a related 1.7-day orbital period.

Studies[3,4] of this first binary X-ray source to be detected reveal that the emission from the system is complex but involves variable emission of X-ray and optical photons with modulations, differing with wavelength, on time scales of 1.24 s, 1.7 and 35 days, the first two being attributed to the pulsar and binary orbit respectively. The origin of the 35-day modulation is less clear. It has been related to the 1.7-day orbital period[5], although many theories have been based on the precession of the rotation axis or the obscuration of our view by a periodically moving disk. The pulsar period has been observed to spin up at a rate, averaged over 11 yr, of about 10^{-13} s s^{-1}. Recent measurements of the X-ray pulse period have been made by the Hackucho and Tenma satellites[6]. In two observations in June 1982 and May 1983, the X-ray data show periods of 1.2377870 s and 1.23778873 s respectively, agreeing broadly with the long-term trend for the pulsar to spin up, but with large and opposite signs of period derivative: $(+7.4 \pm 5.9) \times 10^{-13}$ s s^{-1} and $(-3.6 \pm 1.3) \times 10^{-13}$ s s^{-1} respectively. Optical and IR measurements[7] made in July 1982, a date between the X-ray observations, suggested that the period could be much longer, perhaps as long as that noted in 1972. There is thus recent evidence for a large excursion of the pulsar period from the long-term trend, which is consistent with the indications for the behaviour in 1982–83 suggested by the signs of the X-ray period derivatives. Data on the pulsar period for the past 11 yr are summarized in Fig. 1.

Observations of Her X-1 were made on 17 April 1983 using the array of four telescopes of the Dugway VHE γ-ray facility[8,9]. The telescopes have an energy threshold of ~1,000 GeV, an aperture of ~2° FWHM and a sensitivity (for a 3σ detection of pulsed emission lasting for 10 min) of $\sim 3 \times 10^{-10}$ cm^{-2} s^{-1}. The initial observation comprised four drift scans, each lasting 40 min. The time of arrival of each recorded light flash was corrected to the Solar System barycentre. Assuming that the source of any γ rays is located at the same part of the system as the X-ray source, the times were corrected to the binary focus using the X-ray orbital ephemeris[10].

Emission of VHE γ rays from Her X-1 was noted in the April observation as a marked outburst in the count rates of our telescopes during one of the four drift scans, which were taken in ideal sky and stable experimental conditions. The excess for the total time that Her X-1 was in the field of view of the telescope was $14 \pm 7\%$. The data indicated that the activity lasted for ~3 min, during which an enhancement of $33 \pm 10\%$ in the gross count rate occurred (see Fig. 2*a*). The events during this outburst were periodic with the characteristic X-ray pulsar period (Fig. 2*b*). Because the expected light curve is unknown at these energies, the general Rayleigh test[8] for the strength of the first harmonic of a periodicity was used. The inherent sampling resolution of the periodicity is ~3 ms, rendering redundant the reduction of the orbit to the focus and preventing the measurement of an accurate period. The probability of this periodicity arising by chance is 4×10^{-4}, with an estimated first harmonic periodic content of 39%. The excess periodic γ-ray counts detected in the drift scan are therefore practically 100% pulsed. The profile of the pulsed component through the scan is depicted in Fig. 2*c* as the per cent pulsed in 3-min sliding sections. (The apparent narrowness of the peak in Fig. 2*c* compared with that in Fig. 2*a* is due to the nonlinear relation between chance probability and per cent pulsed signal.)

The increase in count rate and periodicity were shown both by those events triggering a single telescope, and independently by those events in which two telescopes detected the Cerenkov light pool from the same (perhaps higher energy) primary. The excess occurred ~5 min before the object was in the centre of the field of view of the telescope. Inter-detector fast timing indicates that the pulsed events in the excess arrive from a direction ~1° below the centre of the field of view coinciding with the position of Her X-1. There is therefore, in one of four drift-scans, a $>3\sigma$ count rate excess for 3 min from Her X-1, which is periodic at a $>3\sigma$ level at the X-ray periodic. Allowing for the degrees of freedom, the overall statistical significance of this single observation is 7×10^{-5}. The light curve for those events in the 3 min of activity is shown in Fig. 3 and has the

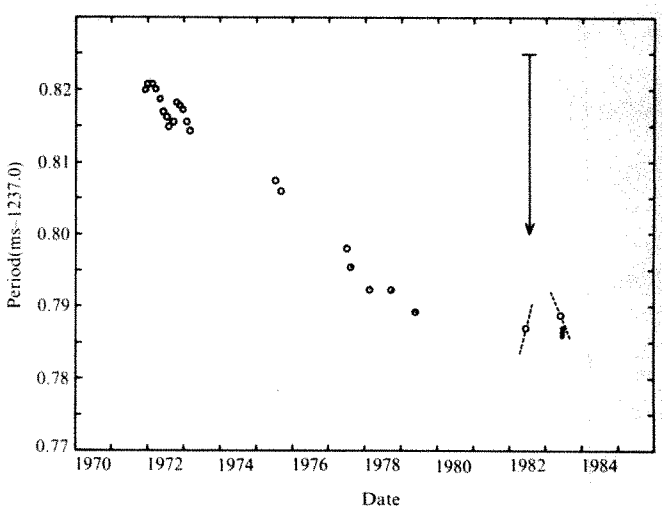


Fig. 1 The period of the Her X-1 pulsar. ▮, Present value derived from the July observation; earlier values are from refs 6, 7. Dashed lines indicate the measured period derivative in X-ray observations.

characteristic broad single peak seen in X rays[11] (with a width about 30%). The peak γ-ray flux during the burst of activity is 1.2×10^{-9} cm^{-2} s^{-1}. The outburst occurred at phase 0.76 in the 1.7-day period and 35 days before a detected turn-on in X rays[6].

Following the 17 April 1983 outburst, Her X-1 was tracked during 3–13 July 1983 and 7–11 October 1983. No further large count-rate excess in a 3-min interval similar to the April event was seen. The July data show an overall periodicity at the 2σ level. Although contributing little to the statistical significance of the detection, these data taken over a prolonged interval allow a test of the accurate Tenma period. The 2σ effect was noted within the sampling range of the extrapolated period from the 1983 May Tenma X-ray measurement (1.2377873 s) and the detected period is indicated on Fig. 1. The time-averaged flux of γ rays is $0.84\pm0.4\%$ of the background cosmic ray flux, corresponding to a flux of $(3\pm1.5)\times10^{-11}$ cm^{-2} s^{-1} for energy >1,000 GeV.

We conclude that Her X-1 is emitting pulsed VHE γ rays sporadically with chance probability of 7×10^{-5} and a peak flux of 1.2×10^{-9} cm^{-2} s^{-1} above an energy of 1,000 GeV at a time in April 1983 when, according to the Tenma satellite data on 22 May 1983, the X-ray turn-on in the 35-day cycle may reliably be expected. The short outburst occurred at phase 0.76 in the 1.7-day orbit when the pulsar in the binary system was moving almost directly away from the Earth. X-ray turn-on has been observed to occur around phase 0.2 or 0.7 in the 1.7-day cycle every 35 days. The optical emission is also strongest at these same two phases[12]. The shortest known time for turn-on is <700 s, being the interval between successive X-ray observations. It is thus possible that the X-ray turn-on is accompanied by the emission of a burst of VHE γ rays lasting ~3 min. If this were the case, an X-ray turn-on took place on 17 April 1983 08.44 UT.

The July 1983 data indicate at the 2σ level that a time-averaged flux of 3×10^{-11} cm^2 s^{-1} exists at the precise pulsar period according to a contemporaneous X-ray measurement (1.2377873 s), if we assume that the VHE γ rays originate with the X rays and make the appropriate allowances for the 1.7-day orbit. A conservative estimate of the probability of chance occurrence of the April event and the overall July emission is 5×10^{-5}.

The VHE γ-ray data were taken when unusual behaviour of the temporal features of the X-ray and optical components was reported [5,7] and when the X-ray flux was decreasing. The time-averaged VHE γ-ray luminosity is 2×10^{35} erg s^{-1} (assuming a differential spectral slope of −3 and distance 5 kpc), which is ~0.5% of the X-ray luminosity including soft X rays.

We know of no previous attempt to detect VHE γ rays from this object. Flux limits[13] on other X-ray sources suggest typical limits of 10^{-10} cm^{-2} s^{-1} at an energy of ~1,000 GeV. The only other (and much more distant) X-ray binary source to be detected in VHE γ rays is Cygnus X-3. The emission from Cyg X-3 peaks at 10^{-9} cm^{-2} s^{-1} during short intervals of the 4.8-h binary period. The duration of the intervals of emission detected during drift scans vary from 15–30 min (ref. 14) to 1 h (ref. 15) (although these durations may be maximum values as they are similar to the duration of the drift scans) and they occur about 0.3 of a 4.8-h period before the X-ray minimum. Continuous observation of the object suggest that the emission may be as short as 3 min (ref. 9). In addition there have been indications that the strength of this 4.8-h periodic emission in both X rays and VHE γ rays

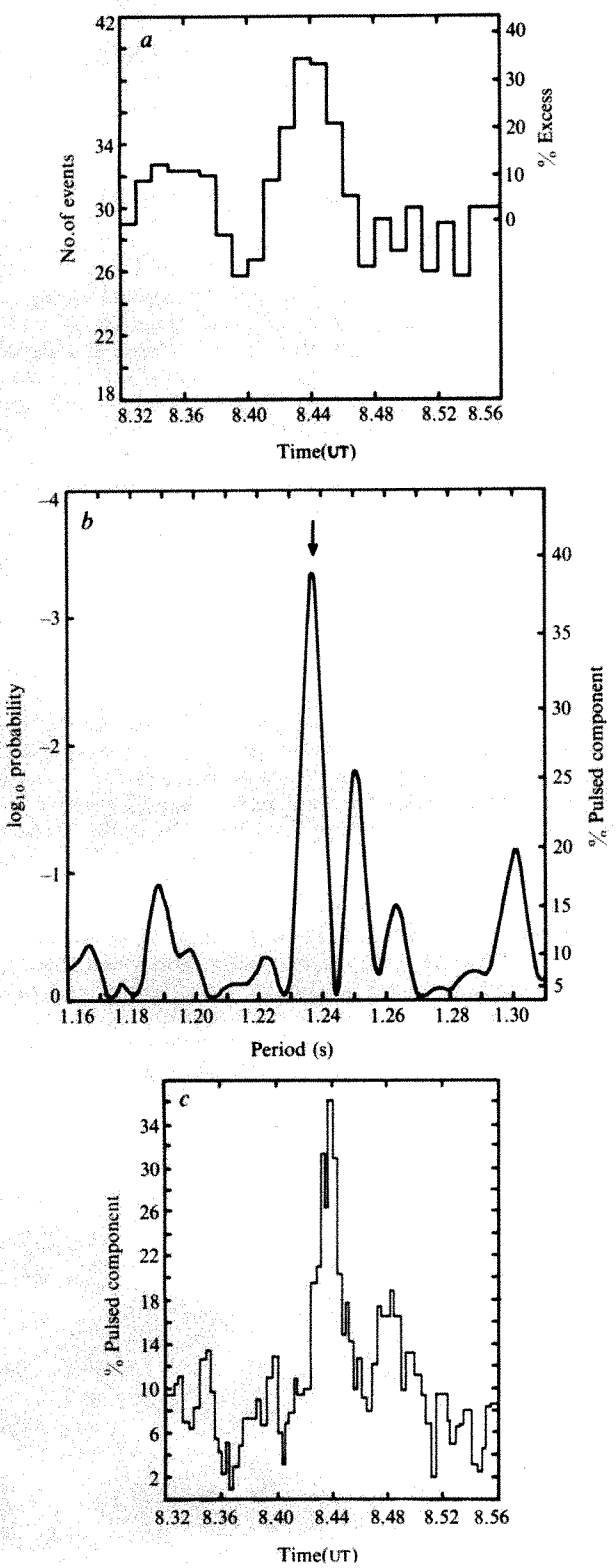


Fig. 2 *a*, The profile of the count rate per minute averaged over 3-min intervals during the observation on 17 April 1983. *b*, The probability of chance occurrence, using the Rayleigh test, and the first harmonic of the pulsed component of the events in the 3-min burst. The arrow marks the predicted period[6]. *c*, The profile of the periodicity (trial period 1.237 s) during the drift scan.

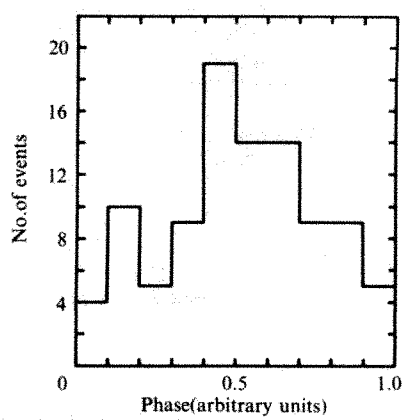


Fig. 3 The light curve for the VHE γ rays from Her X-1 using the trial period of 1.237 s. The data are from the 3 min of the outburst on 17 April 1983.

is itself modulated with a 34.1-day period. There is thus a resemblance between the VHE γ-ray properties of Cyg X-3 and Her X-1, although a pulsar period remains to be detected for Cyg X-3.

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Extraterrestrial platinum group nuggets in deep-sea sediments

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Iron cosmic spheres, composed of iron oxides surrounding a FeNi metal core, were discovered more than a century ago during the voyage of the HMS *Challenger*[1]. That the spheres originated by atmospheric melting of meteoritic metal is supported by a variety of evidence, including elemental composition[2], the presence of the metastable oxide wustite[3], similarity to analogues produced in the laboratory[4] and content of ^{53}Mn produced by exposure to cosmic rays[5]. We report here a previously unrecognized property of the spheres. The most common spheres larger than 300 μm do not, in act, contain FeNi metal cores, but instead contain a micrometre-sized nugget composed almost entirely of platinum group elements. These elements appear to have been concentrated by the oxidation of molten meteoritic metal during atmospheric entry. This process is critically dependent on the relative abundance of oxygen in the atmosphere, and the first appearance of the nuggets in the geological record may provide a marker indicating when the oxygen abundance attained half of its present level.

The spheres used in this study were collected with a magnetic collection sled (the Cosmic Muck Rake), towed across two locations on the ocean floor 1,000 km east of Hawaii[6]. The platinum group nuggets (PGN) were first identified as 5–10-μm diameter spheres in sectioned iron spheres (Fig. 1). A survey of 110 such polished sections, 300–500 μm in diameter, revealed four PGNs. We then mounted 11 300-μm iron spheres and sequentially polished completely through them, inspecting them at 1–2 μm depth intervals. Solitary PGNs were identified in seven of the spheres; additional nuggets may have been plucked out during polishing. None of the PGN-bearing spheres contained FeNi metal cores. From statistical analysis of these results we conclude that half or more of the spheres >300 μm contain a single PGN, that less than half contain the previously described large FeNi metal cores and a smaller fraction contain no metal at all.

The analyses of 10 nuggets shown in Table 1 and Fig. 2 were obtained by energy dispersive X-ray analysis in an analytical scanning electron microscope (SEM). This approach was chosen in preference to the electron microprobe so that the beam could be precisely located on the nuggets. Pure element standards were used and ZAF corrections were made to determine absolute concentrations. The *L* lines were used for elements heavier than Ni and peak integrals were determined by the 'super ML' multiple least squares fitting procedure[7]. The accuracy of this method for the moderately overlapping lines of Ru, Rh and Pd was confirmed by tests on synthetic spectra that closely matched those of the samples.

The analyses show that the nuggets are exotic alloys of group VIII refractory siderophile elements. More than 70% of the mass of a typical nugget is composed of Os, Ir, Pt and Ru. As seen in Fig. 2, five of the nuggets have unfractionated chondritic compositions for the platinum group elements. The mass of these elements in a nugget is consistent with formation by efficient concentration from a sphere of meteoritic metal similar in size to the host oxide sphere. Four of the nuggets have systematic depletions that correlate well with volatility; especially conspicuous are large depletions of Ni and Pd. One nugget is enriched in the more volatile platinum group elements.

A conceivable origin for the nuggets is that they are preserved relics from parent meteoroids and resemble platinum group nuggets found in carbonaceous chondrites[8,9]. The nuggets reported here, however, are much more abundant and are generally larger than those of similar composition found in meteorites. The strongest argument against this source is that PGNs are never found in stony cosmic spheres. In our examination of polished sections of 1,000 stony (chondritic) cosmic spheres in the 300 μm to 1 mm size range, no PGN was observed.

The most likely origin of the PGNs is that they are the natural product of the heating and oxidation of small meteoritic metal particles during hypervelocity entry into the atmosphere. As the metal melts and begins to oxidize, elements more noble than iron remain in a rapidly shrinking metal bead surrounded by a growing shell of immiscible molten iron oxide (Fig. 3). If oxidation is not extreme, the sphere retains a relatively large Ni-enriched metal core. Nickel enrichment by this process is observed in deep-sea spheres[10] and also in spheres associated with terrestrial impact craters[11,12]. With more complete oxidation, most of the iron and nickel leaves the metal core and the result is a PGN. None of the platinum group elements is oxidized because of the low partial pressure of oxygen at the altitudes (70–90 km)[13] where the spheres are molten. Oxidation of Os can occur at this pressure ($< 10^{-5}$ atm for O_2) only if the temperature is below 600 °C (ref. 14); by the time the sphere is this cool, the nugget has been encased in solid iron oxide.

If heating is not severe (for example, due to low entry angle or velocity), the comparatively volatile elements like Rh, Ni and Pd are retained and a chondritic abundance pattern like the flat curves in Fig. 2 can be preserved. Extreme cases of heating result in vaporization of Ru, Pt, Rh, Pd and Ni and the product is a highly fractionated Os, Ir rich nugget, as seen in the lower



Fig. 1 Scanning electron micrograph of an 8-μm platinum group nugget in a polished section of an iron type cosmic deep-sea sphere. The host sphere is composed of magnetite and wustite.

Table 1 Analyses of 10 platinum group nuggets

	83H45	S114	83I4	S-81	83H4	83I8	83I9	83A13	83A21	83I10
Fe	5.7	6.8	6.9	5.6	6.1	6.9	9.9	7.0	4.3	5.6
Ni	9.2	13.3	10.6	2.8	2.7	17.7	56.1	25.3	4.0	1.9
Ru	21.4	19.5	23.0	16.9	19.7	17.2	7.5	12.9	19.7	15.0
Rh	3.9	5.0	6.3	0.9	—	4.0	2.0	3.4	1.4	—
Pd	—	1.2	5.1	—	—	0.5	5.8	3.9	—	—
Os	18.6	15.0	7.4	34.2	29.9	13.6	5.5	12.0	26.0	41.4
Ir	15.6	11.9	7.2	25.9	29.4	13.5	5.4	9.9	26.0	35.2
Pt	25.8	25.7	31.6	12.0	10.0	24.7	10.3	20.6	18.5	1.8
Total	100.2	98.4	98.1	98.3	97.8	98.1	102.5	95.0	99.9	100.6

curves of Fig. 2. Temperatures above 2,400 °C are required to produce significant Ru vaporization[15,16] during the 5 s of peak heating that typical small particles experience near altitudes of 80 km (ref. 13). Significant heating above this temperature would result in vaporization of the oxide shell and the final product would be a bare nugget composed mostly of Os and Ir. Although these bare nuggets have not been observed, they may be more common than those embedded in oxide shells. The Pt/Ru/Rh-rich nugget (the highest curve in Fig. 2) cannot be produced by volatile loss from a source of chondritic composition. Unlike the other nuggets, this one must have originated from a source that has a Pt/Ir ratio higher than chondritic, possibly a III A,B iron meteorite[17]. One remarkable aspect of PGN formation in iron spheres is that the Pt group metals are efficiently concentrated by factors of $>10^5$ above chondritic on a time scale of a few seconds.

We conclude that typical half-millimetre particles of meteoritic metal are severely heated and oxidized during entry into the atmosphere and that these processes concentrate the refractory platinum metals into a small bead $\sim 10\ \mu m$ in size. As described elsewhere[18], there is evidence that most of the iron cosmic spheres are actually not melted iron meteoroids but droplets produced by reduction in carbon-rich chondritic particles undergoing pulse heating at the top of the atmosphere. If this is the case, then a considerable fraction of the Os and Ir currently being accreted by the Earth could be in the form of PGNs. If this process occurs during major inputs of extraterrestrial material into the atmosphere, for example, at the end of the Cretaceous[19], PGNs would form in enormous numbers. The nuggets should be quite stable to alteration and might survive as unique physical markers, while other debris weathers beyond recognition.

The formation of PGNs in iron cosmic spheres requires essentially total oxidation, a process that is only barely possible with the present abundance of oxygen in the atmosphere. Assuming that an impacting air molecule transfers all of its momentum to the decelerating meteoroid, then the particle will decelerate to 13% of its entry velocity after it has encountered an air column whose mass is twice the particle mass. Below this velocity the particle will not remain molten and PGN formation cannot occur. This simple momentum transfer calculation shows that during the time that a small iron meteoroid is strongly heated by atmospheric friction, it collides with a total number of oxygen atoms that is less than twice the number of iron atoms in the particle. Accordingly, we predict that iron cosmic spheres that formed before the abundance of oxygen in the atmosphere reached half its present level could not be totally oxidized during entry and should not contain PGNs. The study of PGNs in iron cosmic spheres recovered from dated stratigraphical layers may provide a means of determining when in the Earth's history the oxygen abundance rose to half its present level.

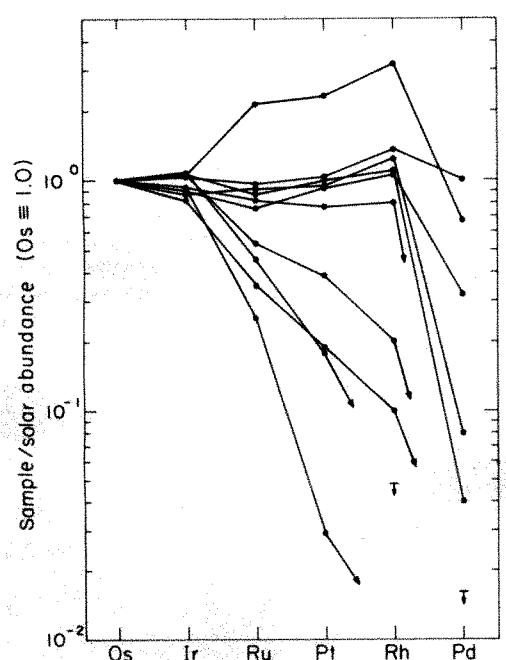


Fig. 2 Abundances of the 10 platinum group nuggets in Table 1 are normalized to the solar composition data of Anders and Ebihara[20]. The data were not directly normalized to CI chondrites because Rh abundances have not been published for carbonaceous chondrites. The elements are plotted in order of increasing volatility from left to right. Temperatures required to produce vapour pressure of 1 torr range from 3,500 °C for Os to 1,900 °C for Pd. This vapour pressure produces vaporization at a rate of the order of 5×10^{-3} cm s^{-1} for a pure element[19,20]. Arrows descending to the right indicate abundances below the indicated detection limits for Rh and Pd.

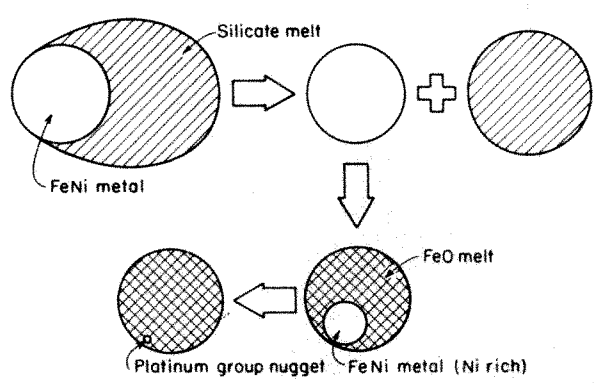


Fig. 3 The most common sequence of events that produces iron cosmic spheres containing platinum nuggets. An interplanetary dust particle melts during atmospheric entry and forms the composite spheroid of molten silicate and metal shown in the upper left. Due to their large density differences, the metal and silicate fractions separate by inertial forces forming a metal sphere and a silicate sphere[18]. The metal sphere develops a rapidly growing oxide shell and siderophile elements more 'noble' than iron concentrate into the shrinking metal core. When oxidation is nearly complete, most of the iron and nickel leave the core, resulting in a small nugget of nearly pure platinum group elements. Continued strong heating may vaporize the oxide shell, leaving only the nugget.

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Evidence from core-reflected shear waves for anisotropy in the Earth's mantle

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Seismologists have begun to recognize that the upper mantle at depth is anisotropic and that seismological data are not fully explicable if anisotropy is ignored[1–5]. On entering an anisotropic zone a plane shear wave splits into two orthogonally polarized phases[6]; an effect observed by several researchers[7], notably by Ando *et al.*[5,8,9]. The near vertical ScS wave, an S wave reflected once at the core–mantle boundary, is one of the most suitable phases to study the phenomenon of shear wave birefringence because it is isolated from other phases and because its particle motion is nearly horizontal and thus free from S to P conversion. The split ScS shear waves travel with different velocities in the anisotropic zone and consequently there is a time delay between their arrival at stations. My aim was to determine this time delay and the azimuth of polarization using the ScS records of two horizontal components from the Japanese islands for a deep shock beneath the Sea of Okhotsk. From the total of 41 stations 34 show a polarization of the maximum velocity phase in the NNW–SSE direction with an average time advance of ~0.8s from the minimum velocity phase. The relevant anisotropic zone seems to be deep-seated.

The particle motion of the ScS wave before it enters an anisotropic zone must satisfy two conditions for the splitting phenomenon to be well observed. First, the particle motion should not be closely parallel to one of the principal axes of anisotropy as little shear wave splitting occurs in such cases. Second, from the focal mechanism point of view, the particle motion must have a large amplitude. The earthquake studied is a rare event that meets these two conditions at almost all the Japanese stations (see Table 1).

The hypocentral parameters of this earthquake are given by ISC as follows: date, 29 January 1971; origin time, 21 h 58 min 03.2 s; epicentre, 51.69° N, 150.97° E; depth, 535 km (pP–P); magnitude, $m_b = 6.0$. We used the data from the three-components seismographs with a natural pendulum period of ~5 s (courtesy of S. Mori of JMA) at 41 stations of the Japan Meteorological Agency (JMA). The epicentre and the station distribution are shown in Fig. 1. The ScS rays are nearly vertical incident to each station because of their closeness to the epicentre. The original paper speed of the JMA seismograms was either 3 cm s^{-1} (smoked paper) or 6 cm s^{-1} (ink writing). All the ScS records of horizontal components were enlarged to an equivalent paper speed of 12 cm s^{-1} and digitized every 0.2 s with an interval of 20 s. The digitized records were then corrected for the armlength of the pen recorder. Figure 2 shows several examples of the resultant horizontal traces; three methods were used to analyse them.

(1) Two horizontal records were synthesized into a particle motion diagram. In the absence of anisotropy the particle orbit was expected to be linear. After passage through an anisotropic zone, however, the particle motion would start with a movement along the polarization azimuth of the maximum-velocity phase and end with a movement along the azimuth of the minimum-velocity phase. The locus in between was expected to be approximately elliptical for a long-lasting signal. As seen in Fig. 2, the initial swing of the observed particle motion was generally directed towards the south-southeast. This swing was then followed by a counterclockwise elliptical rotation. It was difficult to identify the last swing of the ScS motion because of the coda waves. In some cases, however, a last swing towards the east-northeast was seen for example, at URA, ISN, KYO. The particle motion diagrams thus offer a diagnostic demonstration for the existence of anisotropy in the Earth with its maximum velocity polarized in the NNW–SSE direction.

(2) Two horizontal seismograms were rotated in a clockwise direction. An appropriate rotation would resolve the ScS wave

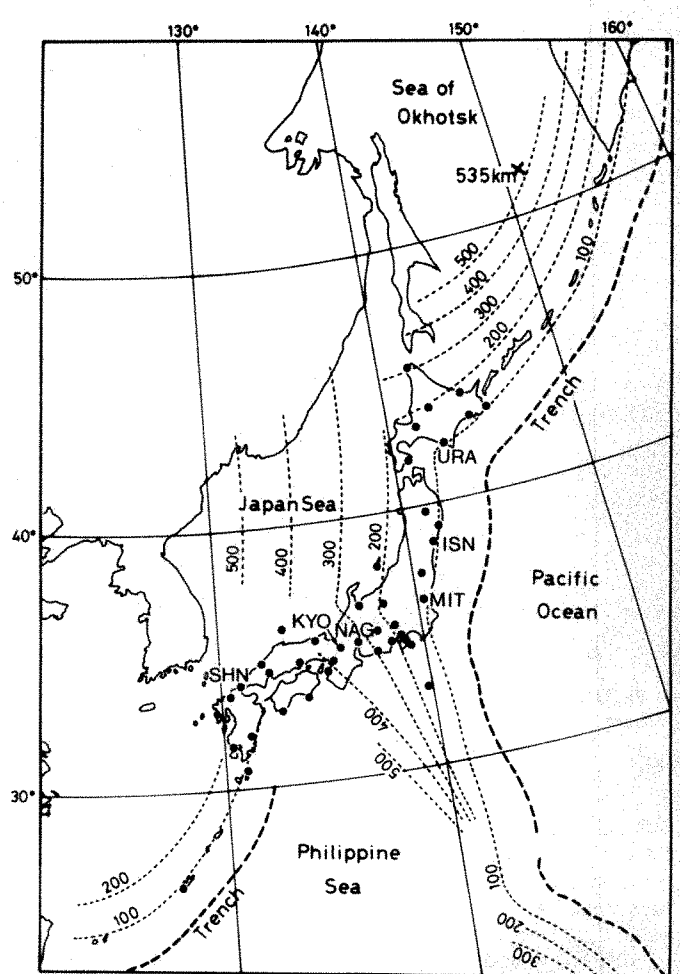


Fig. 1 Epicentre (×) and seismographic stations (●) used in this study. Letters indicate the six stations which appear in Fig. 2. Isodepth contours of deep earthquakes are also shown.

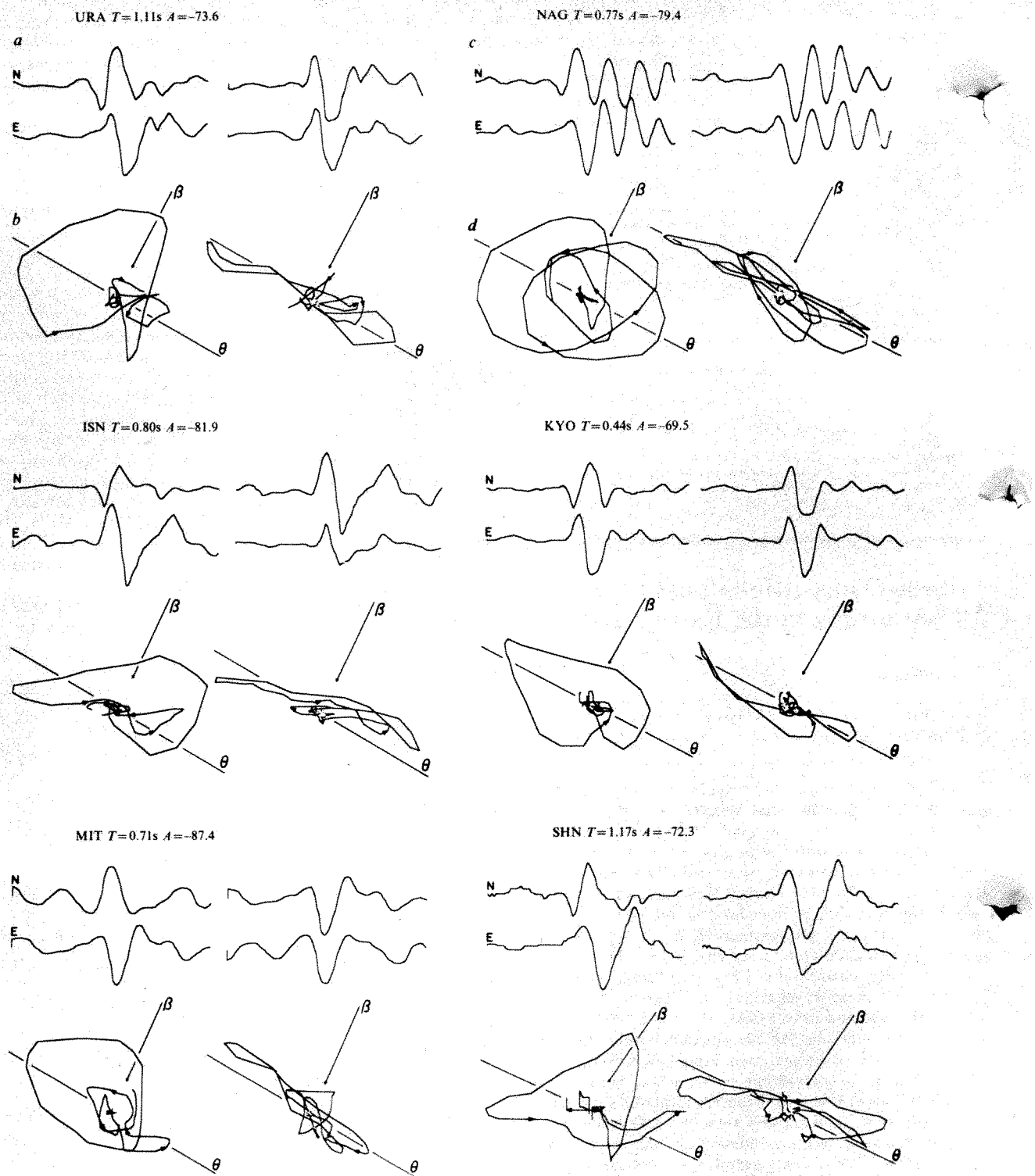


Fig. 2 ScS seismograms at six selected stations (see Fig. 1 for locations). The sampling time is 20 s from the beginning to the end of each trace. *a*, Original north–south and east–west seismograms. *b*, Original particle motion diagrams. β Indicates the incident azimuth of ScS wave and θ the direction of ScS motion expected from the focal mechanism[10]. The expected ScS motion is nearly transverse. *c*, Anisotropy-corrected seismograms obtained by a clockwise rotation A and a relative timeshift T. *d*, Anisotropy-corrected particle motion diagram.

into two orthogonally polarized phases whose wave-forms must be similar to each other. To find an appropriate azimuth of rotation a correlation was taken between the two rotated seismograms. Values for the rotation azimuth φ and the time lag τ yield the maximum correlation and were used to give the axes of anisotropy and a time delay between two polarized phases. (3) Two horizontal seismograms were rotated clockwise and then relatively shifted in time. Appropriate rotation and time shift would show a linear orbit in the resultant particle motion diagram. A straight line was fitted to the particle orbit of rotated and time-shifted seismograms. The rotation azimuth φ and the time shift τ that give the best straight-line fit were, in general, consistent with those obtained by the second method, but did not coincide exactly. A set of φ and τ values which were in better agreement with the qualitative result from the first method were adopted as the final solution.

Figure 2 shows several examples of the seismograms rotated by an angle φ and relatively shifted by a time τ, and are thus corrected for anisotropy. Their waveform correlation is much better than that of the original north–south and east–west seis-

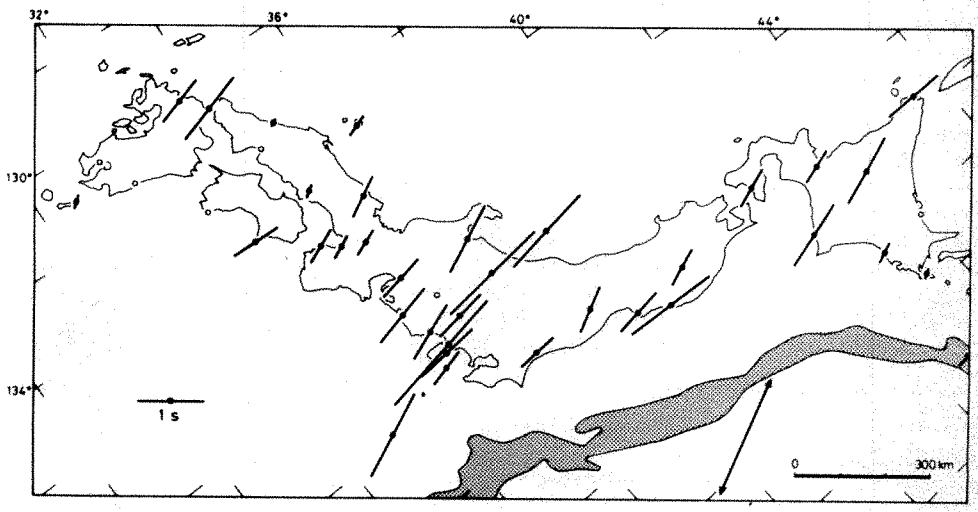


Fig. 3 Polarization azimuth of maximum-velocity phase of ScS wave and time delay between maximum- and minimum-velocity phases. Arrow indicates the azimuth of maximum velocity for horizontal propagation of P waves through the oceanic lithosphere[1].

mograms. Figure 2 also shows the particle motion diagrams corrected for anisotropy. Again the orbital linearity is much better than that of the uncorrected particle motion diagrams. As pointed out by Ando *et al.*[8,9], the linear trend of the corrected particle motion must agree with the direction of the ScS motion predicted by the P wave nodal plane solution. The direction of the ScS motion is calculated for the nodal plane solution given in Table 2 of ref. 10. The direction so calculated is attached to each of the particle motion diagrams corrected for anisotropy; the agreement is very good. The average of the azimuthal differences between the observed trend and the predicted one is $4 \pm 12°$ (35 stations, see Table 1).

The result of the analysis is summarized in Table 1 and is shown in Fig. 3. The uniformity in polarization azimuth is remarkable. Out of 41 stations, 34 show a polarization of the maximum-velocity phase in the NNW–SSE direction. These stations are distributed over all the Japanese islands (Fig. 3). Only NAH, the most distant station from the epicentre, shows a polarization in the east–west direction. If this station is excluded, an average polarization azimuth of (N17° W–S17° E) ± 10° is obtained for the maximum-velocity phase with an average time advance of 0.8 ± 0.4 s from the minimum-velocity phase. A time difference of 0.8 s corresponds to a velocity difference of 4–5% in an anisotropic zone with a vertical extent of 100 km. The velocity difference would be reduced to a half if both the downgoing and upgoing ScS waves pass this anisotropic zone. For the remaining six stations I could not obtain the result with confidence for the reasons outlined in Table 1.

I conclude that there is an anisotropic zone in the ScS ray paths from the hypocentre to the Japanese islands. It is impossible to locate the site of anisotropy from the present analysis. It would, nevertheless, be rather surprising if the upper mantle above the deep seismic zone were to be so uniformly anisotropic over the whole Japanese islands in a variety of tectonic settings. In fact, Ando *et al.*[8,9] observed that the anisotropic nature above the deep seismic zone in Central Honshu, Japan, varies considerably even on a horizontal scale of 100 km. The observed uniformity in polarization azimuth seems to indicate that the relevant anisotropy is deep-seated. For example, the anisotropy may reside in the descending slab of lithosphere. If this is the case, the ScS waves encounter the anisotropic zone at least once near

Table 1 Results of the earthquake analysis

Station	Δ (deg)	β (deg)	A_c (%)	θ_c (deg)	θ_0 (deg)	φ (deg)	τ (s)	Method
WAK	8.8	41	81	117	146	180	1.0	R
ABJ	8.9	28	83	120	*	*	*	*
NEM	9.1	22	84	121	107	152	0.2	C
KUS	9.8	25	84	120	110	158	0.3	C
ASA	9.8	33	83	118	95	160	1.1	R
SAP	10.8	34	83	118	133	162	0.6	R
URA	11.0	28	84	119	115	164	1.1	C
HAK	12.1	32	84	118	123	160	0.7	C
MRK	13.8	26	85	119	121	158	0.6	C
OFU	14.2	24	86	120	125	185	1.5	R
ISN	14.9	24	86	120	106	172	0.8	R
FKS	15.8	25	86	119	104	151	0.7	C
AIK	16.3	29	85	118	104	170	1.5	R
MIT	17.0	23	87	120	130	177	0.7	R
NGN	17.6	27	86	116	109	174	1.8	R
TOY	17.9	29	86	117	96	154	1.1	R
KOF	18.3	25	87	119	121	173	0.9	R
AJI	18.7	23	87	119	130	176	1.0	R
MIS	18.7	24	87	119	112	170	1.8	C
IID	18.7	26	87	118	*	*	*	*
OSH	18.9	23	88	120	129	165	0.6	R
SHZ	19.0	25	87	119	104	159	1.0	R
NAG	19.3	27	86	118	119	169	0.8	C
HMM	19.5	25	87	118	103	167	1.1	R
TYK	19.9	31	85	116	120	150	0.6	R
SAI	19.9	34	84	115	112	159	0.4	C
KYO	19.9	29	86	117	118	159	0.4	R
HIJ	20.3	20	89	121	111	157	1.4	R
OSA	20.3	28	86	117	109	156	0.4	R
WKY	20.9	28	86	117	118	157	0.6	R
OKA	21.0	31	85	116	103	147	0.2	C
HMD	21.6	33	84	114	110	153	0.1	C
HIR	21.9	32	85	115	*	*	*	*
MRT	22.1	28	86	116	110	182	0.8	R
SHN	22.9	33	84	114	102	162	1.2	R
ASZ	23.0	29	86	116	*	*	*	*
FKK	23.5	33	84	114	113	163	0.8	R
MYZ	24.4	30	86	115	†	†	†	†
KAG	25.1	31	85	115	†	†	†	†
TAJ	25.6	29	86	115	93	140	0.2	C
NAH	31.0	29	87	115	101	91	0.7	R

Δ, Epicentral distance. β, Station to epicentre azimuth (from north clockwise). A_c, Relative ScS amplitude expected from focal mechanism. θ_c, Direction of ScS motion expected from focal mechanism. θ_0, Direction of ScS motion observed after anisotropy correction. φ, Polarization azimuth of maximum-velocity phase. τ, Time delay between two polarized phases. C, Correlation analysis of waveform. R, Regression analysis of particle motion.

* Particle motion difficult to interpret.

† Signal amplitudes of NS component too small.

the source and in most cases once again beneath Japanese stations (Fig. 1). The oceanic lithosphere seaward of the Kurile–Japan trench is strongly azimuth-dependent for horizontal propagation of P waves, the direction of maximum velocity being N25° W–S25° E[1]. Note that this direction approximately coincides with the polarization azimuth of the maximum-velocity phase of near-vertical ScS wave. If the oceanic lithosphere consists primarily of olivine crystals whose *a*-axes are aligned in the NNW–SSE direction, both the P and S wave observations could be explained (see ref. 9). Of course, this argument is not valid, if the source of shear wave splitting were located below the depth of olivine–spinel transition.

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Subduction regression and oceanward migration of volcanism, North Island, New Zealand

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In the North Island of New Zealand, calc-alkaline igneous eruptions commenced in the early Miocene and continued into the Holocene in the central Taupo and Egmont areas, but no comprehensive synthesis exists to explain either the volcanic sequence or the regional structural setting. New data, reported here, from K–Ar ages and K_2O–SiO_2 indices for magma source depths indicate an oceanward migration pattern for volcanism, from north-west to south-east towards the Hikurangi Trench. Movement of this volcanic front throughout the Neogene and Quaternary was controlled by subduction zone regression[1] which followed rapid, shallow, non-volcanic Oligocene underplating of the island. In areas of accelerated subduction retreat, tensional tectonism caused widespread foundering of the Mesozoic greywacke basement.

Regional calc-alkaline volcanism in the North Island of New Zealand, of early Miocene–Holocene age, is confined to an area north-west of the main axial range of basement Mesozoic greywackes. Two geographically and petrologically distinct systems of eruptive rocks trend NW–SE parallel to the length of the island (Fig. 1) and contain contrasted calc-alkaline suites: basalts, andesites and rare dacites in the West Belt; and andesites, diorites, dacites, rhyolites and rare basalts in the East Belt[2,3]. The two belts lie on opposite sides of the Stokes Magnetic Anomaly System[4] which may represent the trace of an ancient convergence zone separating older, thicker (Palaeozoic) crust on the west from younger, thinner crust on the east. The NW–SE elongation of the volcanic belts has previously been regarded as an alignment of eruptive centres above a subduction system that flowed under the North Island from NE to SW, forming a Miocene–Pliocene interface between the Pacific and Indian plates[5,6]. Subsequently, this inference has not been supported by the age and orientation of magnetic spreading lineaments in the South Fiji Basin[7] or by the distribution of preliminary K–Ar dates within the volcanic belts[8]. Moreover, Quaternary calc-alkaline eruptive rocks are distributed along a line of volcanism which trends NE–SW from Taupo Volcanic Zone to Egmont

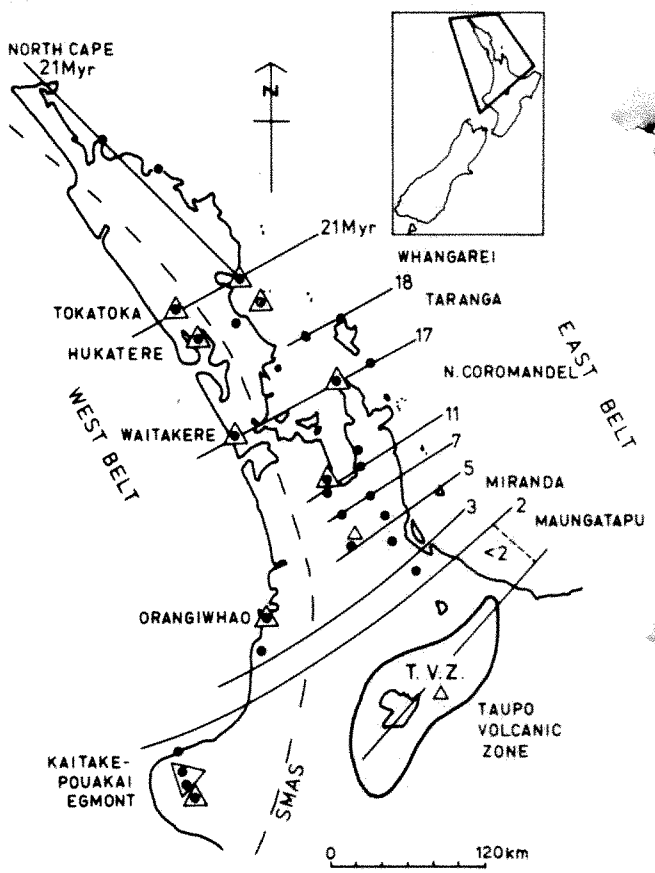


Fig. 1 The northern part of North Island, with the Stokes Magnetic Anomaly System (SMAS) separating the West Belt of calc-alkaline volcanism (Tokatoka to Egmont) from the East Belt (North Cape to Whangarei, and Whangarei to Taupo Volcanic Zone). ●, ○, including the boundary line for Taupo Volcanic Zone (TVZ), provide an indication of the distribution of calc-alkaline volcanic centres in North Island and also give specific locations for K–Ar ages (total of 93 dates) from which the time-lines were determined. △, with place names, are localities for K_2O–SiO_2 estimates of magma source depths shown in Fig. 2.

(Fig. 1) and is almost orthogonal to the East and West Belts, the related modern subduction system, dipping to the north-west, is well defined from seismic data and it originates at the Hikurangi Trench where the Pacific plate descends at an initial shallow angle (~15°) below the edge of the (North Island) Indian plate and then steepens to 50–60° below Taupo–Egmont[6,9].

Additional K–Ar ages for andesites, diorites and dacites within both volcanic belts have provided time-lines (Fig. 1) which represent the oldest dated rocks, and thus the initiation of volcanism, at eruptive centres throughout the northern North Island, and two orientations are apparent.

(1) In the northernmost part of the island, a NW–SE 21-Myr time-line lies along the eastern side from North Cape to Whangarei. Andesites in this zone are unique within the North Island by containing garnet or high-pressure igneous mineralogies, and by having a close association in the field with ophiolite massifs; their origin is believed to belong to a brief magmatic event which accompanied ophiolite obduction at ~23–25 Myr, at the end of Oligocene seafloor spreading in the South Fiji Basin[7,10].

(2) Time-lines with NE–SW orientation record southeastward migration of calc-alkaline volcanism over a distance of 320 km from Whangarei (East Belt)–Tokatoka (West Belt), with oldest ages of 21 Myr, to the Quaternary volcanoes of the Taupo Volcanic Zone (East Belt) and the Egmont group (West Belt). The parallelism of the time-lines and their progressive younging from north-west to south-east are evidence for continued migra-

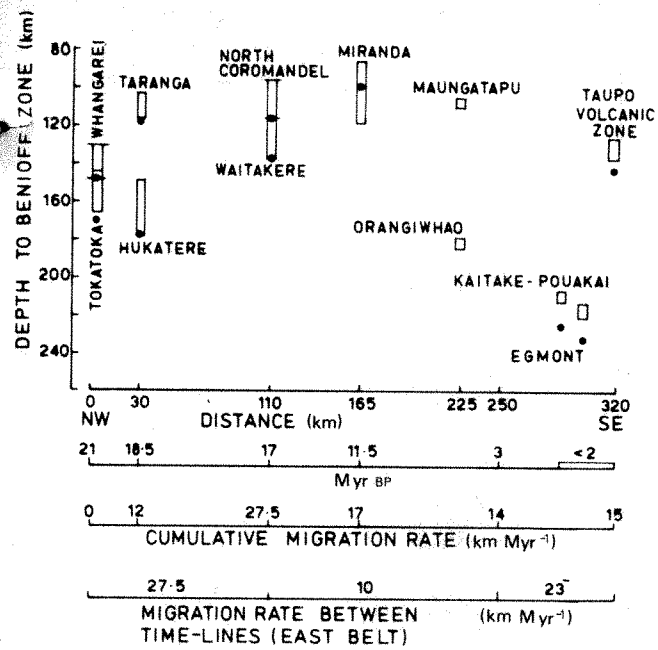


Fig. 2 K_2O–SiO_2 estimates of depth to magma sources, plotted against NW–SE distance from Whangarei–Tokatoka (21 Myr time-line) to Taupo Volcanic Zone–Egmont (<2 Myr) as measured along the East Belt. Rectangles show the total estimated depth range at the centre of the Benioff zone based on the K_{55} and K_{60} method[11]; ●, the maximum estimated depth to the inferred base of the Benioff zone using the $K_{57.5}$ method[12]. The depth shown for Maungatapu is a minimum (using only K_{55} data) and for Orangiwhao is a maximum (using only K_{60} data).

tion of the subduction profile which currently underlies the Taupo–Egmont volcanic centres, with a north-west polarity. Thus, from early Miocene to present day, a mobile volcanic front has accompanied the southeastward retreat of a magma-generating subduction surface (Benioff zone) which developed as a result of oceanward regression of a precursor shallow underplating system that originally must have extended north-west from the Hikurangi Trench for about 650 km inland to the Whangarei–Tokatoka line.

By the potash–silica method, estimates of depths[11,12] for generation of the andesites (Fig. 2) indicate that East Belt lavas consistently originated at shallower levels than those of the West Belt where the greater depth for magma sites, all lying to the west of the Stokes Magnetic Anomaly System, may be related to the presence of a thicker, older sialic basement. Estimation of magma source levels from K_2O–SiO_2 indices is validated particularly for Quaternary lavas of the Taupo Volcanic Zone (124–142 km) and the Egmont volcanoes (206–232 km) where calculated depths lie within the seismically defined profile of the underlying modern Benioff zone[13]. For calc-alkaline suites in the East Belt, shallow origins are indicated also by the general rarity of basalts and by active crustal contamination of magma sources in producing extensive rhyolites and regional hydrothermal metalliferous alteration[14,15]. When compared with this evidence, $^{87}Sr/^{86}Sr$ initial ratios[8,15] seem to be anomalous[12,16], with higher values in the apparently shallower-sourced East Belt (basalts = 0.7041–0.7044; andesites rarely = 0.7035–0.7045, commonly = 0.7050–0.7065) than in the West Belt (basalts = 0.7030–0.7038; andesites = 0.7040–0.7055). The main anomaly is not the isotope ratios, but rather that mantle-derived magmas have failed to react appreciably with the Palaeozoic, more radiogenic crust of the West Belt.

The time-lines for 3 and 2 Myr develop a more westerly trend where they cross the West Belt because of the combined effect of greater depths for those magma sources and the configuration of the regression path for the subduction zone (Fig. 3); these youngest time-lines thus make an angle of ~20° with the clearly defined regional alignment of Holocene volcanic centres in the Taupo Volcanic Zone (East Belt). In view of this geometrical relationship, the older time-lines (21–5 Myr) similarly could have been divergent to the strike of the subduction surface which, at all stages of retreat, possibly approximated the trend of the Taupo Volcanic Zone.

Southeastward movement of the volcanic front, as measured along the East Belt by time-lines 21, 17, 3 and <2 Myr, shows that the rate of migration was not regular (Fig. 2). Initially, at the commencement of subduction regression, migration between time-lines 21 and 17 Myr proceeded at a rate of 27.5 km Myr^{-1} and the intervening region was subjected to pronounced tensional tectonism that produced widespread foundering of the greywacke basement (Fig. 4) and a deep basin for concurrent early Miocene flysch sedimentation[17]. In contrast, the following period of relatively slow migration at 10 km Myr^{-1}, between time-lines 17 and 3 Myr, traversed a region where the greywacke basement was extensively blockfaulted but not notably depressed, so that it persisted as a shallow marine shelf for calcareous Miocene–Pliocene sediments. Subsequent acceleration in the migration rate, at 23 km Myr^{-1} for the interval 3–<2 Myr, was again characterized by tensional collapse of the greywacke basement to depths of 5 km and accompanied by voluminous eruptions of silicic calc-alkaline volcanics in the Taupo Volcanic Zone[9,18].

Underplating of the North Island by the Pacific plate, before the early miocene, would have involved rapid shallow

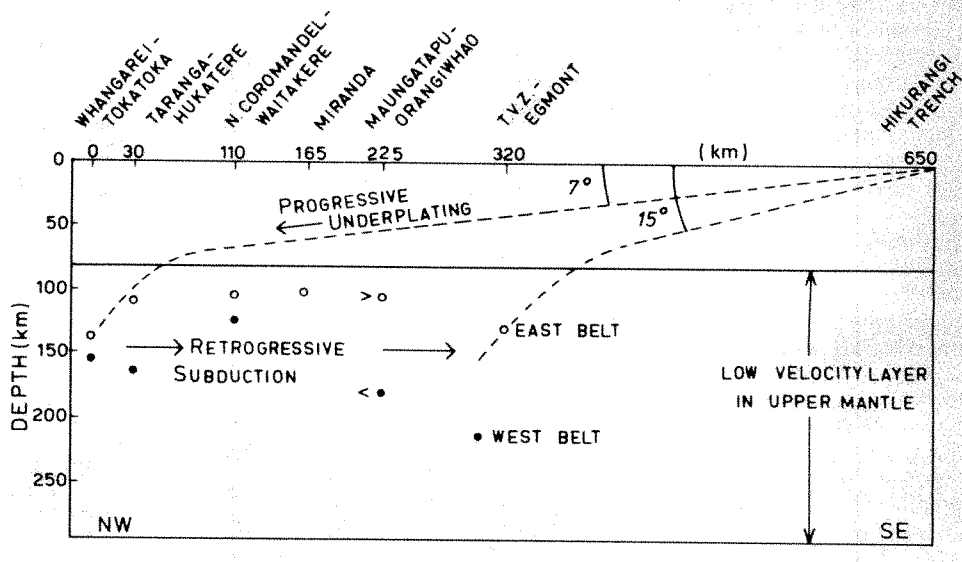


Fig. 3 SE–NW profile along the East Belt from Hikurangi Trench to Whangarei–Tokatoka (21 Myr time-line) with averaged K_{55}–K_{60} estimates of magma source depths for East Belt (○) and West Belt (●). Progressive shallow underplating during the Oligocene was followed by Miocene–Holocene subduction retrogression to the profile of the present Benioff zone[13] beneath Taupo Volcanic Zone and Egmont.

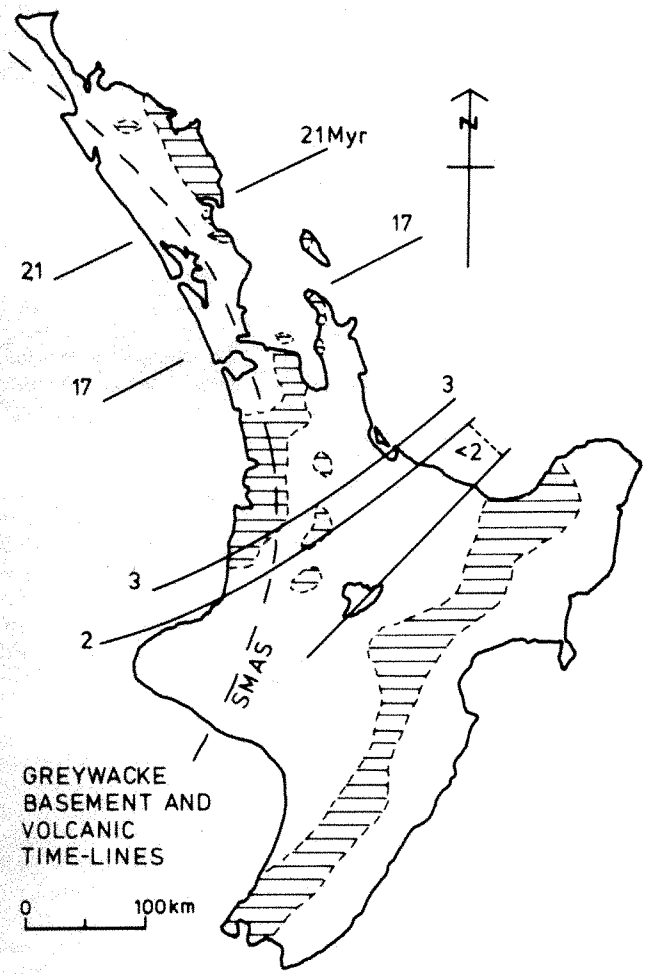


Fig. 4 North Island Mesozoic greywacke basement outcrop pattern, developed by tensional tectonic collapse within regions of accelerated subduction regression between volcanic time-lines 21–17 Myr and 3–<2 Myr.

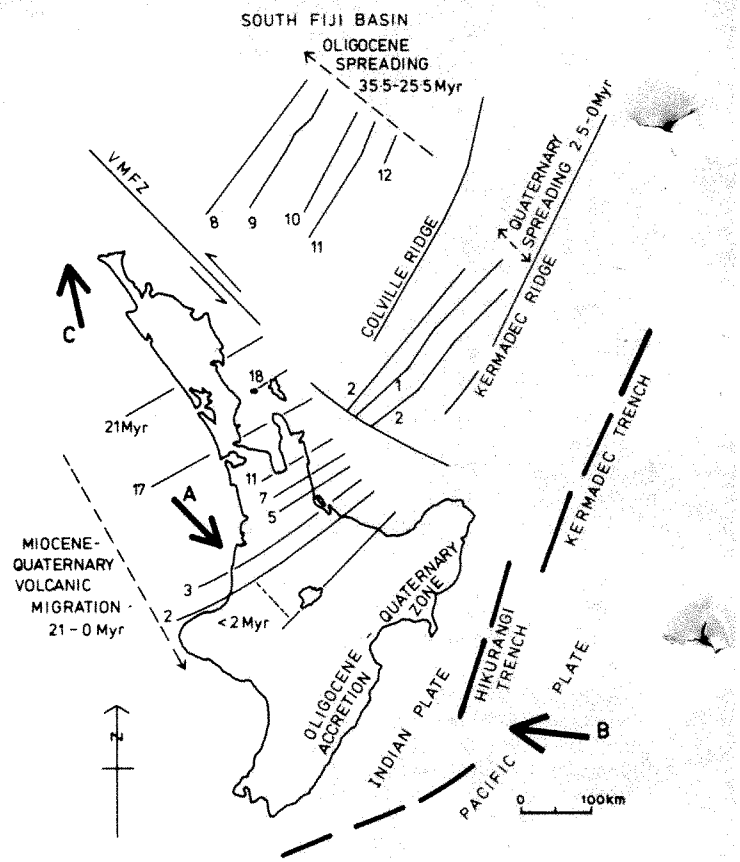


Fig. 5 Late Tertiary and Quaternary lineaments in the North Island and adjacent oceanic area. Oligocene marginal basin spreading (magnetic anomalies 12–8) produced left-lateral displacement along the Vening Meinesz Fracture Zone (VMFZ), and after Neogene quiescence was followed by Quaternary spreading (anomalies 2–1) between the Kermadec and Colville Ridges[7]. Arrow A shows Oligocene motion of North Island (Indian plate) relative to the South Fiji Basin. Arrow B is indicative of various directions within the west quadrant for absolute motion of the Pacific plate from Oligocene (anomaly 13) to Holocene, and arrow C is the present absolute motion vector for the North Island[22,25,26].

subduction not accompanied by volcanic activity for a distance of 650 km from the Hikurangi Trench to the Whangarei–Tokatoka line, a tectonic style which is similar to the amagmatic Laramide event in the western Cordillera of North America[19]. The geometry of the underplating process can be achieved by assuming a low initial trajectory of 7° (Fig. 3) which implies a rapid rate of convergence between the upper plate and the subducting slab, and a buoyant strong mechanical coupling between the two plates[20,21] which is supported by the presence of an accretionary prism of scraped-off Oligocene–Quaternary marine sediments on the landward side of the shallow Hikurangi Trench (Fig. 5). Marginal basin spreading during the Oligocene (35.5–25.5 Myr) caused offset of the North Island towards the south-east, relative to the South Fiji Basin[7], in opposition to the general westerly direction of motion at that time for the Pacific plate[22], thus enhancing the velocity of convergence at the subduction interface. This 10-Myr period of accelerated convergence, with the North Island (Indian) plate overriding the Pacific plate, could have provided the mechanism necessary for Oligocene underplating at a rate of at least 65 km Myr^{-1}. The end of seafloor spreading in the South Fiji Basin (25.5 Myr)[7] was followed by southwestward obduction of ophiolites onto the North Island (25–23 Myr)[10] and by a reduction in convergence velocity for the shallow subducting slab which, with a consequent increase in gravitational sinking rate and a steepening profile[23,24], reversed its mode at 21 Myr from north-west directed progressive underplating to southeastward subduction regression towards the Hikurangi Trench. The patterns for seismic activity[13] and younging of volcanic ages across the modern Benioff zone (Figs 1, 3) suggest that subduction retreat towards the south-east still continues.

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A late Devensian ice-free area and possible interglacial site on the Isle of Lewis, Scotland

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At the time of the last glacial maximum the last Scottish ice sheet has been considered by some[1,2] to have extended to the continental shelf edge (Fig. 1*a*) whilst others[3,4] have suggested that there were ice-free areas in northern Scotland. No radiocarbon-dated stratigraphical evidence has been published to support either interpretation. We present here data from the Isle of Lewis establishing that the shelly deposits from the north of that island are of late Devensian age but which also show that a late Devensian ice margin lay across northern Lewis leaving part of the island ice-free. A peat, possibly of interglacial status, has been found in the ice-free area. The last Scottish ice sheet therefore did not pass beyond the Outer Hebrides and may not even have reached the north of the island chain. This evidence is also of interest to the debate on the relative expansions of the northern and southern margins of the Laurentide and Scandinavian ice sheets at the last glacial maximum[5,6], for it suggests a restricted glacial expansion of the northern margin of the last British ice sheet at the time of its greatest southerly extent.

Detailed mapping at a scale of 1:10,560 and recording of all natural sections in the north of Lewis have confirmed McCann's[7] observations that on part of the north-west Lewis coast, a raised beach occurs at an altitude of ~12 m (local OD)[8]. Along the stretch of coast marked a–a′ in Fig. 1*b* the beach is *in situ* and up to 150 m broad. Frequent cryoturbation structures at the top of the gravels together with solifluction and head deposits that rest on and partly incorporate the beach gravels establish that the beach predates at least one period of periglaciation[4,9]. The beach is best developed along the coast south of Galson and is here termed the Galson Beach. Although composed dominantly of material derived from the local Lewisian gneisses, the beach also contains frequent red and purple sandstone clasts that diminish in abundance southwards. The present study has also confirmed that along section a–a′ of the coast the beach occasionally overlies a till which rests on a wide (up to 150 m) rock platform[4,7,9] at an altitude of ~9 m (local OD)[8].

South-west of Melbost Borve (a, Fig. 1*b*) Peacock[4] noted that the gravels of the Galson Beach were overlain by, or incorporated in, a till and he suggested that the late Devensian limit of Outer Hebridean ice occurred in this vicinity. North-east of South Dell (a′, Fig. 1*b*) the complex shelly glacial drifts can also be observed to overlie Galson Beach deposits. At Bad an Fhithich (BF, Fig. 1*b*) beach gravels (which may be an erratic) have been reported[4,7] to overlie till although at the same locality stratified beach cobbles and sands resting *in situ* on bedrock are truncated by and overlain by till.

Detailed mapping of the shelly glacial deposits indicates that only one phase of glacial activity is represented. Complex glacitectonic structures and large erratics of marine clays and gravels have given rise to Baden-Powell's[10] interpretation of two glacial phases with an intervening period of interglacial marine deposition. These glacial deposits terminate abruptly on their southern margin and this limit can be traced across north Lewis to a major drift ridge up to 30 m high that extends for over 6 km to the south-southeast to reach an altitude in excess of 110 m (Fig. 1*b*). North and east of the ridge are extensive glacial deposits, areas of dead-ice terrain and a sequence of meltwater channels trending north-northwest.

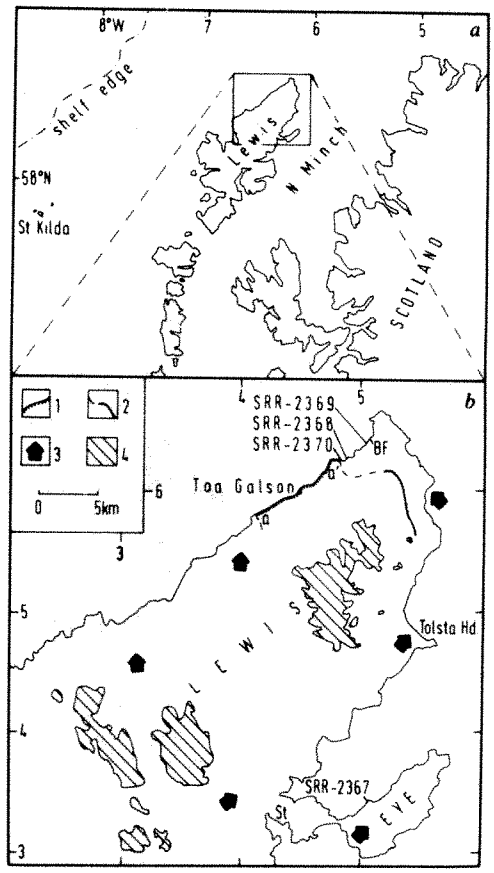


Fig. 1 *a*, Location of Lewis and *b*. *b*, Northern Lewis. 1, Stretch of coast (a–a′) where the Galson Beach is *in situ* and not overlain by glacial deposits; 2, ice margins, continuous line representing the end moraine in north Lewis; 3, generalized directions of last ice movement; 4, land over 122 m (400 ft).

The drift ridge is interpreted as an end moraine of the last ice mass to occupy the north of Lewis and the trend of the moraine, together with the direction of slope of the meltwater channels indicates that the ice had a surface gradient from south-southeast to north-northwest. Abundant purple and red sandstone clasts in the glacial deposits are most probably derived from Mesozoic[11] sedimentary rocks that floor the North Minch close offshore and they cannot be taken as evidence for the presence of Scottish mainland ice which their original attribution to the Torridonian was thought to imply[12]. Indeed, the last ice in this area may have been no more than Outer Hebridean ice recurving round the upland of northern Lewis on to the low ground farther north.

Samples of shell debris from three localities in the middle or base of these glacial deposits (Fig. 1*b*) have been radiocarbon dated (Table 1) and all gave middle Devensian ages. The shell faunas in the glacial deposits are derived from a variety of oceanographic provinces and thus very probably represent a range of ages from the ice-free period before the last ice advance. Hence the 34,000–39,000 yr BP sample ages are likely to result from a mixture of late Devensian and middle Devensian (or older) shells. Amino acid analyses of ice-transported shells from north Lewis suggest a late Devensian age (D. Q. Bowen, personal communication), supporting this interpretation. The evidence therefore indicates that the last glaciation in the north of Lewis was during the late Devensian. Expansion of the Outer Hebridean ice cap during the late Devensian is also indicated by the date on the ice-transported shells from Garrabost on the Eye Peninsula (Fig. 1*b*, Table 1), the last ice movement there as determined by carry of local erratics being from south-west to north-east.

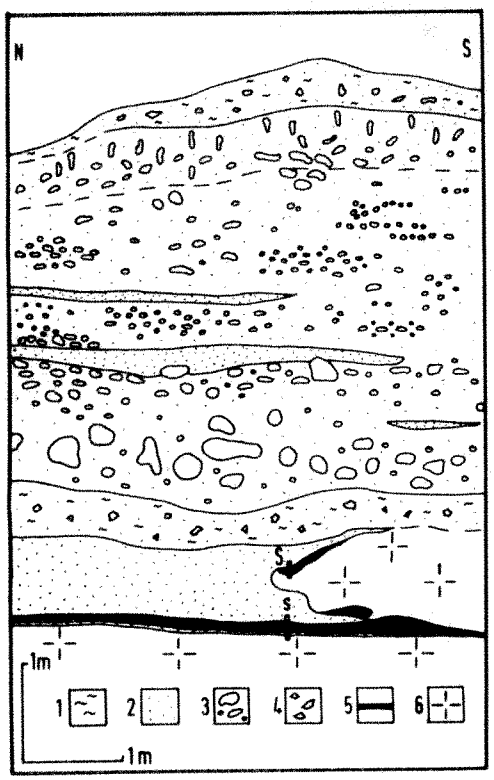


Fig. 2 Section south of Toa Galson. 1, Silt and clay; 2, sand; 3, rounded boulders and cobbles; 4, angular pebbles and gravel; 5, peat; 6, weathered or decomposed bedrock. Note cryoturbated upper ~75 cm of beach gravels. Samples for pollen analysis taken from locations marked S.

The establishment of the latest glacial event in the north of Lewis as being late Devensian in age and the stratigraphical evidence that the Galson Beach underlies or is incorporated within these glacial deposits implies that the stretch of coastline a–a′ (Fig. 1*b*) was ice-free during the late Devensian. A wave-eroded section at the front edge of the raised rock platform ~100 m south of Toa Galson (Fig. 1*b*) is particularly significant in this context, because there a peat that postdates the formation of the raised rock platform and the glaciation of this part of the coast, but predates the deposition of the Galson Beach, has been found. The stratigraphy is shown in Fig. 2.

The peat has been distorted and partly eroded by a slump of decomposed bedrock that is contemporaneous with the overlying medium-coarse iron-stained sands. Here we term the main peat body as the lower peat, Site A, the uppermost disturbed peat lens is the upper peat, Site A and a peat lens that occurs several metres farther south in the section is Site B. Between the iron-stained sand and the beach gravels is a layer of angular locally-derived clasts in a silty-sand matrix that shows minor silt lenses and weak lamination that are typical of solifluction.

Samples for pollen analysis were removed from the peat and adjacent sediments (Fig. 2) and the spectra recorded in the three peat lenses are shown in Fig. 3. In the main peat body (lower peat A) the lower samples are dominated by Gramineae and Cyperaceae but Cruciferae, *Plantago* spp., *Rumex* and *Saxifraga* are consistently represented. An open, floristically-diverse grassland is suggested with wetter areas providing a habitat for the sedges and certain species, possibly, of the Umbelliferae. With continued accumulation of the peat there is a steady increase in Ericaceae (including *Empetrum*) pollen which suggests a progressive change in the local vegetation and in the upper part of the peat the pollen spectra imply an acid heath vegetation interspersed with grassland communities. *Empetrum nigrum* is generally considered to be an oceanic species[14,15] and the change in the pollen spectra may reflect increasingly oceanic conditions with soils becoming leached and base-poor. Throughout the pollen profile there are low percentages of arboreal pollen, principally *Pinus*, although *Picea*, *Betula*, *Alnus* and *Abies* grains were also recorded. These are considered to have been derived by long-distance transfer and, with the possible exception of small areas of *Salix* and *Juniperus* scrub, the environment is interpreted as being essentially treeless.

Plant macrofossils were poorly preserved in the peat, leaf fragments of *Sphagnum imbricatum* being found in the lower part and *Viola* seeds occur throughout (P. Hulme, personal communication). Undifferentiated *Viola* seeds have a wide range of occurrence during the Pleistocene[16], but *S. Imbricatum* is of greater interest. Dickson[17] indicates that in pre-Lateglacial times *S. Imbricatum* has only been recovered from interglacial deposits with the exception of one possibly-derived attribution to the Anglian.

The upper surface of lower peat A is clearly erosional but the basal sample of the overlying sand was found to be polleniferous. This revealed a pollen spectrum very similar to the base of lower peat A. As some, if not all, of the pollen grains in this sample may have been derived from erosion of the peat no environmental change may be interpreted from this single sample. The pollen spectra from upper peat A and from site B (Fig. 3) are very similar to those from the upper part of lower peat A with a strong Ericaceae (and *Empetrum*) component. These analyses confirm that the peat lens was originally more extensive and has been eroded and disturbed by the subsequent events at the site.

A sample was removed for radiocarbon dating from the upper part of lower peat A. The sample was split into three fractions

Table 1 Radiocarbon dates on ice-transported marine shells from Lewis

Site	Laboratory no.	$\delta^{13}C$ (‰)	^{14}C age (yr BP)
West Dell Sands	SRR-2368	(o) +2.3	$37,070^{+970}_{-860}$
		(i) +2.3	$34,470^{+720}_{-660}$
Traigh Chumil	SRR-2369	(o) +1.2	$39,850^{+1270}_{-1100}$
		(i) +1.9	$39,500^{+1270}_{-1100}$
Peicir	SRR-2370	(o) +1.6	$37,320^{+1130}_{-990}$
		(i) +1.4	$35,630^{+1520}_{-1270}$
Garrabost	SRR-2367	(o) +1.6	26,300±320
		(i) −3.0	23,000±230

Pretreatment for all these samples consisted of discarding 25% by weight of the bulk sample by acid hydrolysis with the remaining carbonate being divided into equally sized 'outer' (o) and 'inner' (i) fractions by further acid treatment.

Table 2 Radiocarbon dates on buried peat layer at Toa Galson

Laboratory no. SRR-2365.

Fraction	$\delta^{13}C$ (‰)	$d^{14}C$ (‰)	^{14}C age (yr BP)
(a)	−29.3	−1000.9±0.7	>47,150
(b)	−29.2	−1000.8±0.8	>46,640
(c)	−29.7	−1001.1±1.9	>39,100

Fraction (a) was composed of ≥125 μm material that was acid washed in 2M HCl; fraction (b) consisted of <125 μm material that was soluble in 0.5 KOH; and fraction (c) was the residual <125 μm material following the alkali treatment. Fraction (b) should relate to humic acid contamination and fraction (a) should contain the coarse parts of any modern rootlets that may have penetrated the peat. The acid wash was to remove any carbonates that may have been precipitated in the peat.

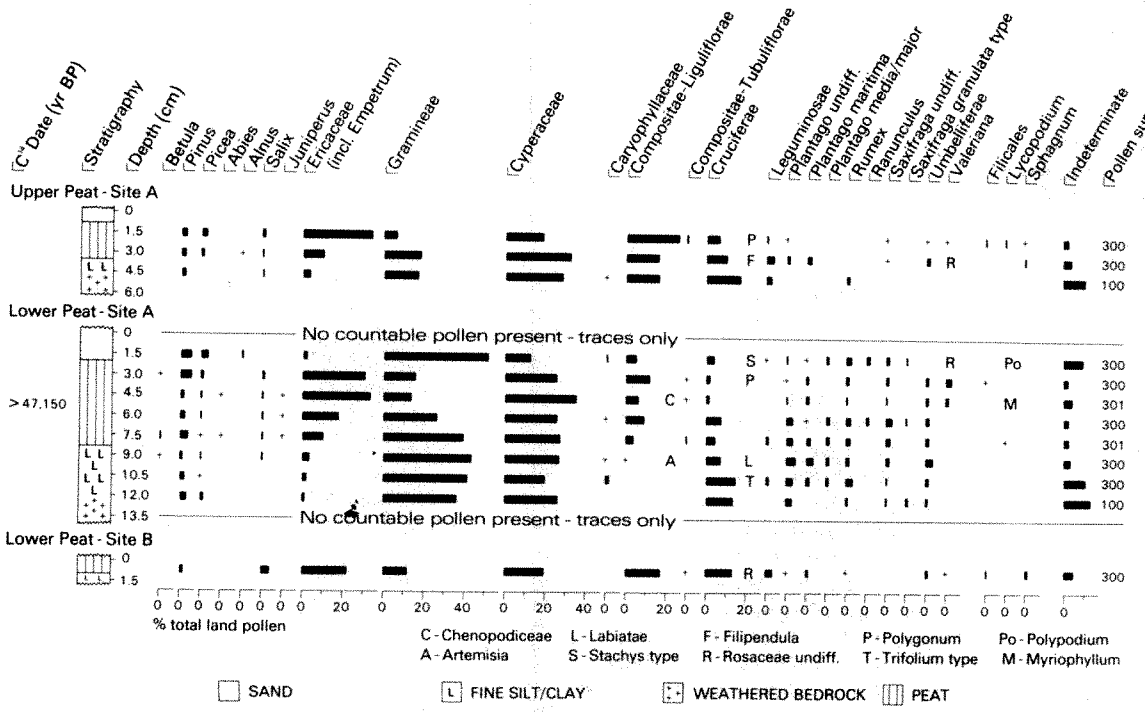


Fig. 3 Percentage pollen diagram from Toa Galson. Samples for pollen analysis were prepared using conventional procedures[13], except that residues were passed through microsieves (aperture 10 μm) to concentrate pollens and spores. Pollen preservation was good in the peat layers but in the minerogenic sediments large numbers of corroded and amorphous grains were found.

following a procedure designed to isolate the possible contaminants (carbonates, organic acids, modern rootlets). Details of the pretreatment and the radiocarbon results are given in Table 2. The agreement between all fractions indicates minimal contamination and a sample age of >47,150 yr BP.

The essentially treeless environment that has typified the northern and western islands of Scotland during the Flandrian and presumably previous interglacials and interstadials poses considerable problems in assessing the status and correlation of pre-late Devensian pollen-bearing sediments. Two other such sites are known from the Outer Hebrides, Tolsta Head[18,19] and St Kilda[20] (Fig. 1). As both these sites are associated with finite radiocarbon ages and have significant differences in their pollen spectra from that of Toa Galson, the sites are not thought to be the same age. Farther afield, however, there are certain similarities between the Toa Galson pollen profile and that from Sel Ayre, Shetland[21] where a vegetational development from grass and fern dominated communities to dwarf-shrub heaths dominated by Ericaeous shrubs, including *Empetrum*, was recorded. The local environment was treeless and the Sel Ayre site was assigned an interglacial age on the basis of inblown tree pollen and tentatively ascribed to the Ipswichian. The progressive changes of vegetation at Sel Ayre were suggested to represent an interglacial cycle in a treeless environment and if this model is correct then the Toa Galson peat may tentatively be assigned an interglacial status. Such an interpretation is in agreement with the occurrence of macrofossils of *Sphagnum imbricatum*, the floristic diversity of the grassland communities, the occasional thermophilous inblown tree species and the radiocarbon age. There is, however, no evidence that allows the site to be ascribed to a particular interglacial.

Models of the last ice sheet showing Scottish ice extending to the continental shelf edge depict the north of Lewis as being covered by 1,000–1,500 m of ice[1,2] but our evidence demonstrates that part of this area was actually ice-free. This implies a relatively restricted late Devensian glaciation along the north-west margin of the Scottish ice sheet, the ice perhaps extending no more than 100 km from the major northern Scottish mainland ice shed. Yet at this time the ice sheet reached the Midlands of England, south-west Wales and southern Ireland. In recent years there has been considerable discussion as to the relative expansions of the northern and southern edges of the Laurentide and Scandinavian ice sheets at the last glacial maximum[5,6]. In contrast to the traditional view of synchronous glacial expansion, a 'minimalist' school has suggested that the northern margins had a relatively restricted glaciation at the time of maximum southerly advance. We suggest that a similar north–south contrast typified the late Devensian British ice sheet and palaeoclimatic models that seek to explain the north–south contrasts of the larger ice sheets must also be capable of explaining a marked climatic gradient across the more southerly British ice sheet.

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Interpretations of the magnetostratigraphy of the Hadar hominid site, Ethiopia

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As part of an investigation to determine the age of the hominid-bearing strata of the Hadar Formation of Ethiopia we have determined the magnetostratigraphy of two separate sections of the strata. Two major zones of reversed polarity occur within the predominantly normally magnetized sequence, one at the top and the other in the middle. There are difficulties with the absolute age determinations of volcanic units within the strata which result in uncertainties in correlating the Hadar magnetostratigraphy with the standard magnetic reversal time scale. The age of the upper part of the section is established by the BKT-2 tuff at ~2.8 Myr (refs 1–4). The K–Ar dating of the Kadada Moumou basalt which lies in the middle of the sequence yields variable results ranging from 3.1 to 3.6 Myr (refs 1–4). Regional geochemical[5] and biostratigraphical[6] studies suggest the younger age for the Kadada Moumou basalt. We show here that this is also the age that results in the most consistent interpretation of the observed magnetostratigraphy of the formation, which is that the Hadar Formation was deposited during the Gauss Normal epoch and that the two reversals represent the Kaena and Mammoth events within that epoch.

The Hadar Formation is exposed in a badlands topography along the Awash River valley to the south-west of the town of Mille (Fig. 1*a*). As the beds of the Hadar Formation are horizontal or dip gently and only minor faulting is present, continuous stratigraphical sections can be sampled along the main wadis. Two major sections were sampled (Fig. 1*b*) along the Kada Hadar drainage (called the camp section) and along the Ounda Hadar drainage (called the Ounda Hadar section).

Details of sampling and laboratory procedure are given elsewhere[2,7]. Samples in the camp section were spaced at 0.5-m stratigraphical intervals, the Ounda Hadar section was sampled at more widely spaced intervals, the lower part with a large and variable interval of up to 3 m and the upper part at a 1-m interval. The natural remanent magnetization (NRM) varied, in the range of 1–50 mA m^{-1}. All samples were progressively demagnetized in alternating fields. The NRM directions of magnetization were variable; normal and reversed directions were found as well as an appreciable number of intermediate directions; the intermediate directions gradually disappeared during the progressive demagnetization. The directions of magnetization of some samples did not stabilize until high fields were applied (50–75 mT, Fig. 2*b*).

The cleaned magnetic declination of the camp section samples are shown in Fig. 2*a* in stratigraphical order with the previously published upper part of the Ounda Hadar section. The data for the camp section differ from previously presented data[2,7] in that alternating field demagnetization was continued to higher fields. The higher field demagnetization better defines the reversed intervals (see Fig. 2*b*). The comparison of the two sections is very favourable in that the fine scale of variation in declination between the two sections matches well. Although there are normal directions of magnetization within both reversals which are quite similar in both sections, their origin is not certain.

There are few radiometric data to tie the observed polarity sequence to the standard magnetostratigraphical scale because there are only two horizons in the Hadar Formation which can be used to establish the age of the formation[1–4]; these are the BKT-2 tuff and the Kadada Moumou basalt. Other volcanic horizons have large detrital components or excess argon is suspected rendering them unsuitable for age determinations.

The age of the BKT-2 is interpreted as 2.88 ± 0.08 Myr, based on several K/Ar age determinations and fission track data from zircons[1–4]. However, the age of a Kadada Moumou basalt is not clear. Repeated K/Ar analyses do not converge on a single age and age estimates show a very strong correlation with petrology. Walter[3] identified three varieties of the basalt identified as A-1, A-2 and B type basalts yielding K/Ar ages of 2.7–3, 3.2–3.4, and 3.6 Myr respectively.

The type B basalt is presently represented by only one hand sample and although the age is probably quite reliable, because it is represented by only a single sample and because its chemistry is different from the A types, it is necessary to consider a possible interpretation that it is a xenolith from an older flow, or that it contains excess argon. There are problems with the type A basalts also, for they show a correlation between observed alteration and age. As might be expected, the A-1 type basalts which are the most altered give the youngest ages. However, Walter *et al.*[4] prefer the 3.6-Myr age for the basalt. Figure 3 shows two possible interpretations of the polarity data as indicated by the various ages which may be attributed to the Kadada Moumou basalt.

The original interpretation[1,2,7] assumes the A-1 age, 2.7–3.0 Myr, for the basalt. The two reversed zones are then correlated with the Kaena and Mammoth events within the Gauss normal epoch. This interpretation is consistent with the reversed magnetization of the Kada Moumou basalt.

A second interpretation (not shown) assumes the A-2 age, 3.2–3.4 Myr, for the basalt. This requires the upper reversed zone to represent the Kaena and Mammoth events. This is inconsistent with the lower reversal, in that the lower reversal which includes the reversed basalt would correlate with the base of the Gauss normal epoch. For this correlation to be true, the Kadada Moumou basalt and lower reversal would have to be the result of remagnetization. There is no evidence of remagnetization of the basalt and consequently this possibility is rejected.

The third interpretation which assumes a type B age for the basalt, 3.60 ± 0.15 Myr, requires the upper reversed zone to contain both the Kaena and Mammoth events. The lower reversal would correlate with the top of the Gilbert reversed polarity epoch. While satisfying most of the polarity data, the extent of the normal section below the reversed horizon poses a problem.

Table 1 Sedimentation rates implied by the different interpretations of the Hadar Formation

Stratigraphical interval	Magnetic interval	Stratigraphical thickness (m)	Interpretation 1 Age range (Myr)	Interpretation 1 Sedimentation rate (cm kyr^{-1})	Interpretation 3a Age range (Myr)	Interpretation 3a Sedimentation rate (cm kyr^{-1})	Interpretation 3b Age range (Myr)	Interpretation 3b Sedimentation rate (cm kyr^{-1})	Depositional environment
Middle KH	Upper reversal	35	2.92–3.01	39	2.92–3.15	15	2.92–3.15	15	Deltaic fluvial
Lower KH Middle DD	Middle normal	40	3.01–3.05	100	3.15–3.40	16	3.15–3.40	16	Deltaic fluvial
Middle DD Upper SH	Lower reversal	25	3.05–3.15	25	3.40–3.80	6	No data	No data	Lacustrine
Upper SH Base	Lower normal	70	3.15–3.40	28	3.80–3.90	70	No data	No data	Deltaic fluvial lagoonal base

To account for the absence of a reversed interval between the Cochiti and Nunivak events either the normal magnetization at the base of the section consists of the Cochiti event (which would require a very high sedimentation rate), interpretation 3a, or the base of the section has been remagnetized, interpretation 3b, which is shown.

The net sedimentation rates through the section can be used to examine the internal consistency of the various age interpretation for the basalt. Table 1 shows the net sedimentation rates for the stratigraphical section implied by the interpretations 1, 3a, and 3b. The sedimentation rates were calculated by dividing the thickness of the magnetozones by the duration of appropriate zone as given by Mankinen and Dalrymple[8]. The thicknesses are approximate in that the thickness of the Hadar Formation is variable in the study area. Most of the variation, however, is due to variation in thickness of the SH unit. The depositional environment of the Hadar Formation is given in the right column of Table 1; details of the development of the sedimentary model are given elsewhere[9,10]. The sedimentation rates in a fluvio–lacustrine environment such as the Hadar Formation may be expected to be variable. However, the broad trends in sedimentation should vary with the sedimentary environment. During periods of fluvio–deltaic activity when large amounts of sediment are being deposited in the nearshore regions and deltaic facies are dominant, then relatively high sedimentation rates are possible (though unconformities could also be present). When there was a major transgression of the lake and lacustrine environments dominated, the sedimentation rate could be expected to be lower.

Interpretation 3a implies a sedimentation rate of only 6 cm kyr^{-1} for the Upper SH to Middle Denon Dora but a minimum sedimentation rate of 70 cm kyr^{-1} for the lower part of the section. While an unconformity could be present which could lower the apparent sedimentation rate, there is no evidence of

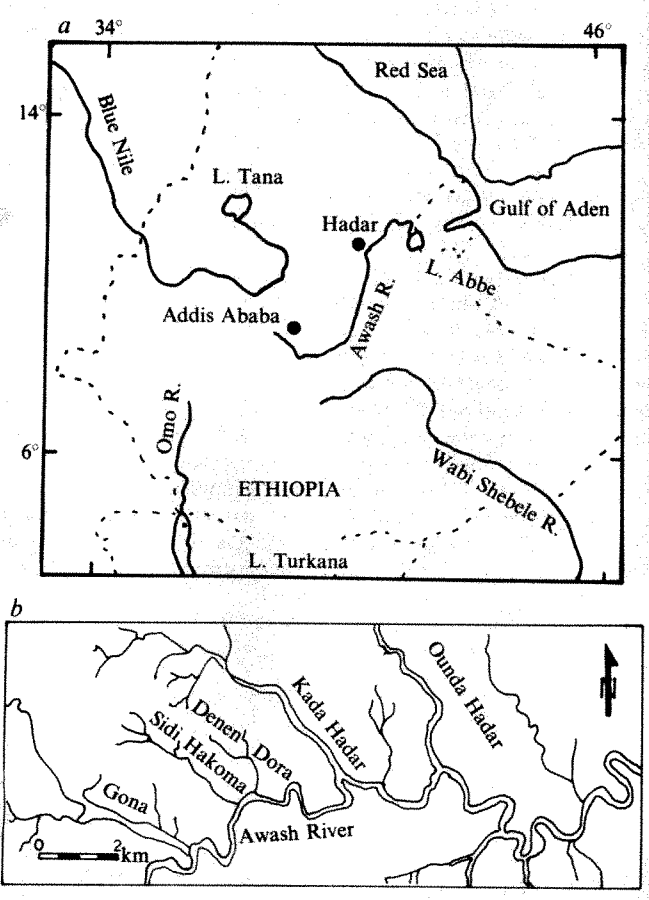
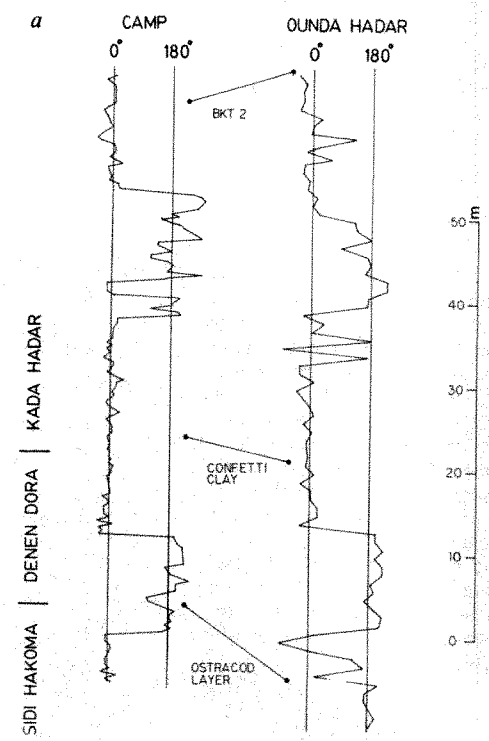


Fig. 1 Setting of the Hadar hominid site. *a*, Location of Hadar with respect to major drainage. *b*, Location of stratigraphical sections with respect to local drainage of the Hadar region.



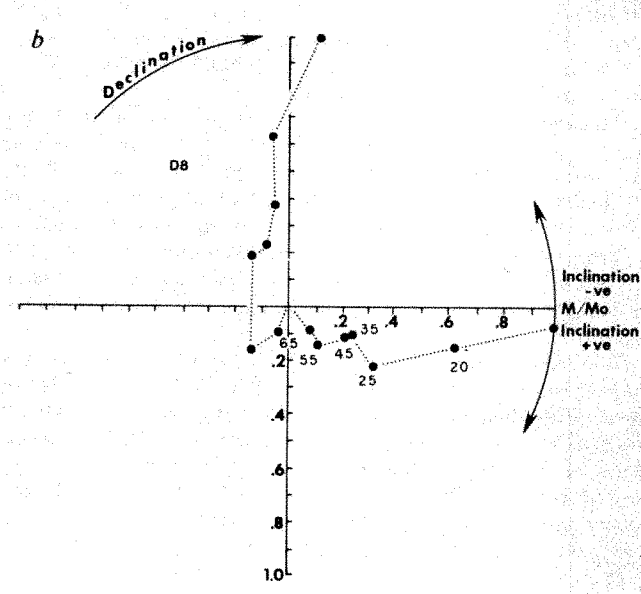


Fig. 2 *a*, Magnetic declinations plotted against stratigraphical position of the upper half of the Hadar Formation. Declinations are plotted from the upper half of the camp and Ounda Hadar sections. The lower half of both sections are normally magnetized. The magnetic directions are cleaned to 500 or 750 Oe. If the samples were still changing direction between 350 and 500 Oe then they were demagnetized to 750 Oe. The previous presentation of the camp section[2,7] was at lower demagnetization stages. *b*, Stable end point diagram of a representative sample of the Hadar Formation. The declination and inclination of magnetization of the sample are plotted against normalized intensity of magnetization at each demagnetization stage. The demagnetization stages are listed in the diagram in mT. The first stage is NRM. A significant change in direction of the sample (a reversal) with high demagnetization fields is shown.

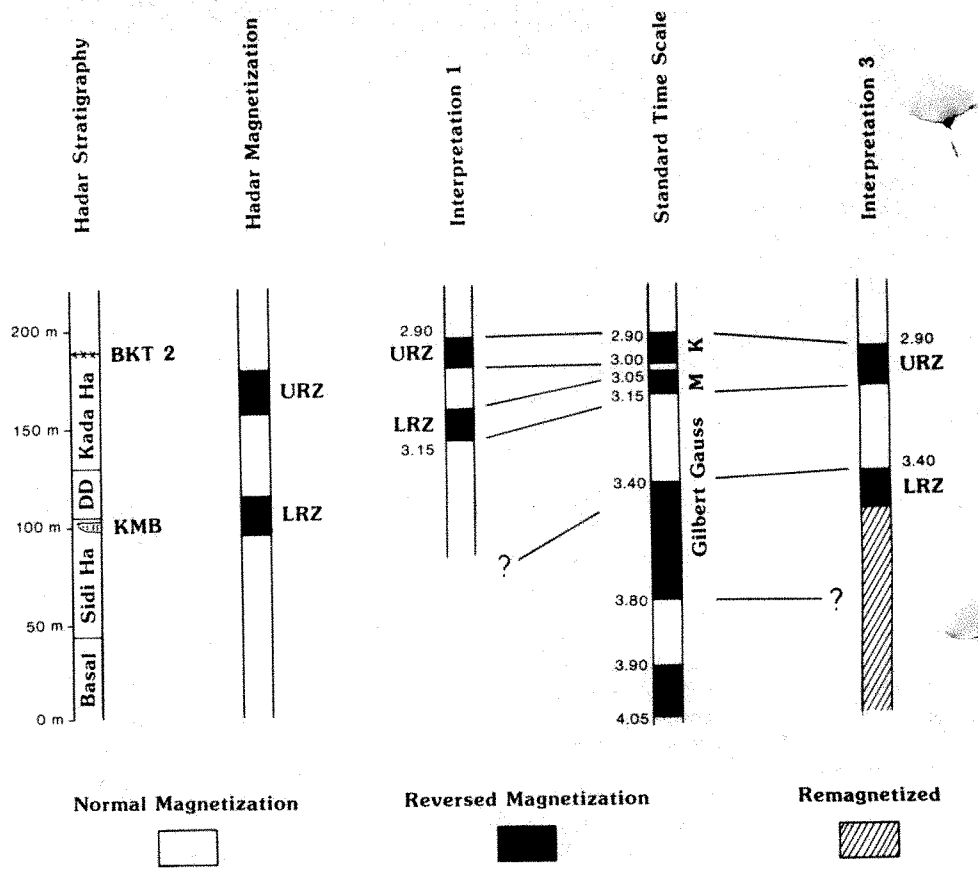


Fig. 3 The various interpretations of the Hadar magnetostratigraphy are shown with the 'standard' magnetostratigraphy and stratigraphy of the Hadar Formation. The standard ages for the Gauss and Gilbert are from ref. 8. K, Kaena; M, Mammoth events. The Hadar magnetization is from this and previous papers. The interpretations are as in the text. The schematic of the Hadar Formation is modified from Walter and Aronson[4]; the thicknesses of the Hadar Formation are variable, the thicknesses noted here are approximately the thicknesses of the members of the 1975 camp section.

a time gap large enough to reduce the rate to that of 6 cm kyr^{-1}. Also, the implied minimum sedimentation rate of 70 cm kyr^{-1} is considerably higher than the sedimentation rate implied for similar facies elsewhere in the section.

Interpretation 1 implies a relatively high sedimentation rate (40–100 cm kyr^{-1} for the fluvio–deltaic periods and low rate (2.5 cm kyr^{-1}) during the lacustrine period. The 28 cm kyr^{-1} rate for the base and SH is a minimum rate and thus not inconsistent with the higher rates for the similar facies higher in the section.

Interpretation 3b implies a sedimentation rate of 15–16 cm kyr^{-1} for the upper part of the section. The sedimentation rate for the lower part is indeterminate due to interpreted remagnetization.

In the Omo Valley of southern Ethiopia and northern Kenya and the basins along Lake Turkana in Kenya there are hominid-bearing fossiliferous strata temporally equivalent to the Hadar Formation. Further constraints on the age of the Hadar Formation can be made by comparison of the Hadar Formation with those formations. Brown[5] and Boaz *et al.*[6] compared the Hadar Formation with the Omo and Turkana facies by geochemical and palaeontological means, respectively.

Brown compared the minor and trace elements chemistry of the Sidi Hakoma Tuff of the Hadar Formation with the Tula Bor Tuff of the Koobi Fora Formation, and its equivalents, Tuff B of the Shungura Formation, and Tuff U-10 of the Usno Formation. They are strikingly similar. He took this to indicate that they were the result of the same volcanic eruption. The correlation is consistent with the magnetostratigraphy. These tuffs are assigned to the lowest normal of the Gauss Normal epoch; thus the stratigraphical correlation implies that the lower reversed zone of the Hadar Formation is the Mammoth event, between 3.05 and 3.15 Myr.

Boaz *et al.*[6] attempted a biostratigraphical correlation of the Hadar Formation with the Shungura and Usno formations. They compared the evolutionary stage of individual genera and species and made a statistical correlation of the Hadar fauna with a set of a faunal assemblages from the age range 2.9–3.15 Myr. They found the Hadar Formation most similar to the Usno Formation, which has the older of the two faunas. They concluded that the biostratigraphy was consistent with the geochemical correlation made by Brown[8]. There are, however, some constraints on biostratigraphy, for the age of the Hadar Formation is from 2.9 to at least 3.15 Myr and perhaps 3.6 Myr. That is, the Hadar age range covers both of the standards compared and although most of the Hadar fauna are from the Denon Dora member, the correlation was based on the full faunal list.

The consistency of the polarity sequence of the two separate magnetostratigraphical sections after cleaning strongly suggests that the sequence is a true record of the magnetic field at the time of deposition of the strata and that there is minimal remagnetization. The age of the upper part of the section is reliably established as about 2.8 Myr. Using that as an anchor point the uppermost reversed zone is correlated with the Kaena event and the middle reversed zone is correlated with the Mammoth event. This is consistent with regional geochemical[5] and biostratigraphical[6] correlations. The net sedimentation rates implied by the correlation are consistent with the A type basalt ages and supports the original[1,2,7] age estimates for the Hadar Formation and its important fossils.

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Adult-obtained pyrrolizidine alkaloids defend ithomiine butterflies against a spider predator

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Ithomiine butterflies, the original distasteful models in neotropical mimicry complexes, show warning coloration and are usually rejected by both vertebrate and invertebrate predators[1–4]. Based on co-evolutionary theory[1,5–7] and the Danainae–cardenolide example[7], this protection has been ascribed to toxic compounds sequestered by larval Ithomiinae from their host-plants, usually poisonous Solanaceae[8–10]. However, although the giant tropical orb spider *Nephila clavipes* cuts out field-caught Ithomiinae from its web[4,11] it readily eats freshly emerged adults. Solanaceae toxins are not found stored in adult Ithomiinae[6,11] and when applied to the palatable[1] butterfly *Biblis hyperia* do not induce rejection behaviour in *Nephila* (Table 1). I show here that the protection of ithomiines against this abundant predator is due to dehydropyrrolizidine alkaloid monoesters and their *N*-oxides, absent from Solanaceae but sequestered by these butterflies as adults from flowers (mostly Eupatorieae) and decomposing foliage (mostly Boraginaceae)[11–13]. These compounds are also important in the reproduction of the Ithomiinae[11,14].

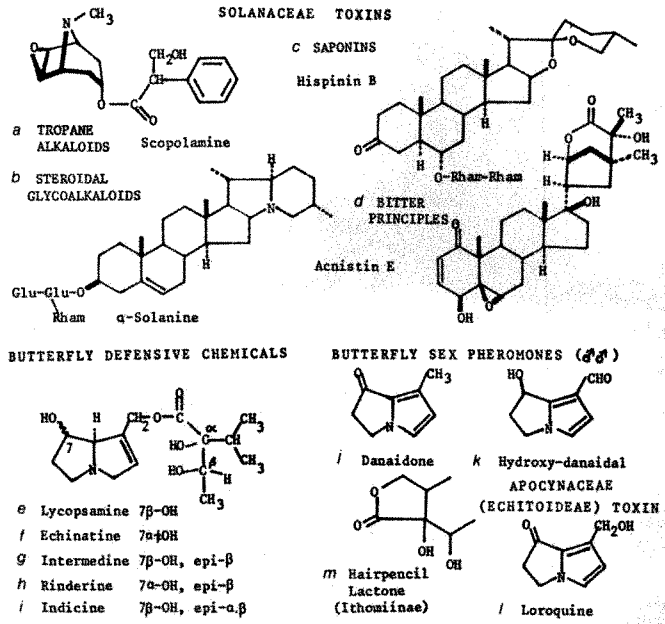


Fig. 1 Representative Solanaceae toxins (*a–d*)[8–11] and Danainae/Ithomiinae defensive and reproductive substances (*e–m*)[11–16,21–24].

Table 1 Chemical analysis and bioassay of Ithomiinae and their foodplants

Ithomiine tribes (*b–l*), representative genera (no of species analysed/total no. in genus) and PA content of individuals in typical populations (average % dry weight; male/female)	Fractions bioassay	Larval foodplants: family, tribe (I–XII or name), representative genera (no. spp. analysed)	Chemical compounds	Fractions, bioassay
		Apocynaceae: Parsonsieae (fresh leaves, roots, stems)		
(*b*) *Tithorea**† (1/2)0.30/0.30;	Ex (+)	*Prestonia* (2), *Peltastes* (1), *Temnadenia* (1)	*eloqr*	Ex, 1, 2 (−)
(*c*) *Aeria*† (3/3)2.3/1.6	Ex (+)	Solanaceae (fresh leaves)		
(*d*) *Methona**†‡ (4/7)0.031/0.026	Ex (+)	XII *Brunfelsia* (2)	*a?no*	Ex (−)
(*e*) *Placidula*† (1/1)0.11/0.037	Ex (+)	II *Datura* (1), *Brugmansia* (1)	*adnop*	Ex, 2 (−)
(*f*) *Melinaea*† (5/10)2.8/1.1	Ex (+)	VI *Solandra* (1); VII *Markea* (*Dyssochroma*) (1)	*as*	Ex, 1 (−)
(*g*) *Thyridia** (1/1)2.7/2.2, *Sais** (1/2)3.6/1.7	Ex (+)	Ia *Cyphomandra* (2), *Lycianthes* (2)	*bcnops*	Ex, 1 (−)
V *Mechanitis**‡ (5/5)1.8/2.6 or 2.8/1.8, *Scada* (12/6)13.4/3.6;	Ex, 2, 3 (+) 1, 4 (−)	*Solanum*, subgenera *Brevantherum*, *Leptostemonum*, *Bassovia* and *Potatoe* (14)	*bcnp*	Ex, 1–4 (−)
(*h*) *Hypothyris**‡ (6/16)2.2/1.5;	Ex (+)			
(*j*) *Dircenna**‡ (3/7)1.7/0.88	Ex (+)			
(*h*) *Epityches*‡ (1/1)6.7/3.4;	Ex (+)	Ib *Physalis* (1), *Acnistus* (1), *Capsicum* (3)	*acdn*	Ex, 1–3 (−)
(*i*) *Ithomia**‡ (4/21)5.4/2.0	Ex (+)	Ia *Solanum*, section *Geminata* (3)	*bcp*	Ex, 1, 2 (−)
(*k*) *Prittwitzia*‡ (1/1)2.8/1.7, *Pteronymia*†‡ (5/41)9.0/3.3;	Ex, 2, 3 (+) 1, 4 (−)	VIII *Cestrum* (2)	*bcops*	Ex, 1, 2 (−)
(*l*) *Mcclungia*‡ (1/1)1.7/1.1, *Pseudoscada*‡ (3/6)4.8/2.2	Ex (+) Ex (+)			
Adult foodplants of Ithomiinae, Danainae and Arctiidae, and larval foodplants of various species in the latter and other moth families as well as some Diptera		Boraginaceae: *Heliotropium* (3) (leaves)	*his*	Ex, 2 (+)
		Compositae: Eupatorieae (leaves) *Adenostemma* (2), *Trichogonia* (1), *Eupatorium* (8)§	*e − i, ors*	Ex, 2, 3 (+)

Complete species lists and analytical results are given in ref. 11. Over 2,500 individual butterflies in Danainae (3 genera, all 6 South American species) and Ithomiinae (32 of 34 Brazilian or 51 neotropical genera, 82 species) were analysed and shown to store PAs. In most individuals, 50–65% of the PA was *N*-oxide and 2–5% dihydropyrrolizine (type *k*), with ketones (types *j* or *l*) also detected. Unspecified amounts of PAs (*e*+*g*), previously found in *Oleria* and *Hypomenitis*, were considered as precursors of pheromone *m*, isolated from male hairpencils of *Ithomia*, *Pteronymia*, *Godyris*, *Hypomenitis* and *Greta*[14]. A total of 42 species of larval foodplants (in 19 genera), extracted and fractionated in parallel with their herbivores, always gave negative assays with *Nephila* except with very heavy loads on the *Biblis* (>10 mg; became negative upon dilution). Over 400 blind bioassays were performed using over 100 *Nephila*, in triplicate (if +) or duplicate (if −), with code-numbered total extracts (Ex) or fractions (1-neutral; 2-alkaloidal; 3-polar; 4-apolar). Letters for chemical compounds refer to Fig. 1, plus *n*-other alkaloid classes, *o*-phenolic glycosides, *p*-pungent oils, *q*-cardenolides, *r*-lower terpenes and *s*-alkaloid *N*-oxides. Ithomiinae and Solanaceae tribe letters and numbers are in approximate evolutionary order[8,10]; note the apparent lack of 'parallel diversification' type co-evolution between the genera of these herbivores and their host-plants[5,10,11].

* Eggs of these species showed from 0.1% (*Methona*) to 9% of dry weight as PAs.

† Mature larvae of these taxa are aposematic in colour-pattern.

‡ Recently emerged adults of 13 species in these 10 genera, of both sexes, reared in the laboratory from eggs or larvae and denied access to PAs, were consumed without hesitation by *Nephila* which in many cases had just cut out a field-captured adult of the same species and sex. Mattocks–Bingley assay[25,26] showed no PAs in these and in many other recently emerged adults.

§ Flowerheads of several abundant Brazilian species of *Eupatorium* contain 1–4% of a single isomer of PA (*k–o*) and the corresponding *N*-oxide, and thus represent important new sources for these compounds, some of which are now in advanced clinical testing as antitumour agents.

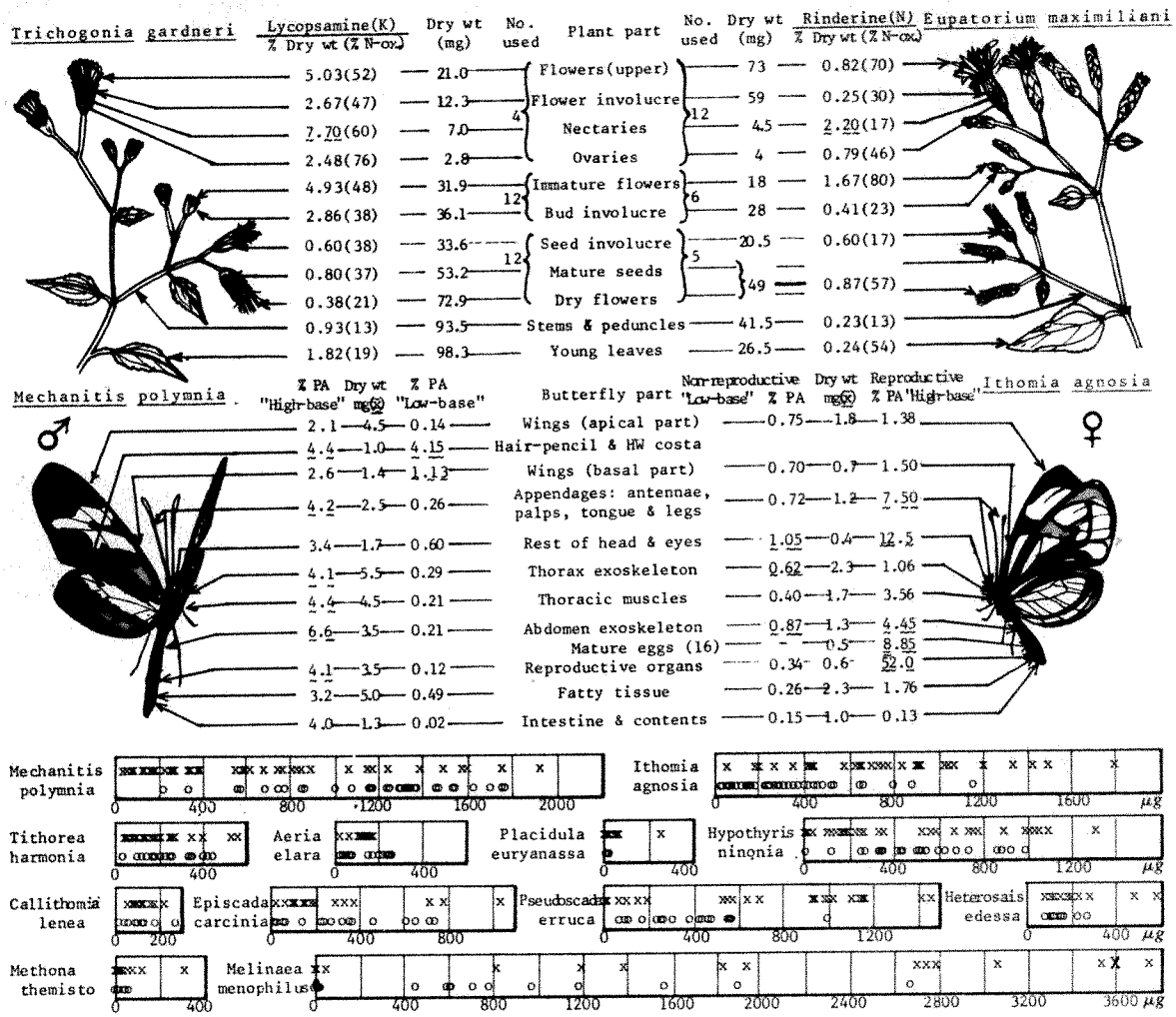


Fig. 2 Distribution of PAs in two species of Eupatorieae commonly visited by Ithomiinae (upper), and (lower) within and between individual adults of typical Ithomiinae (see Table 1 for further data and chemoassay details). High-base non-reproductive and low-base reproductive females of *Ithomia agnosia* were also encountered. The thorax tended to show low *N*-oxide content (<50% of PA) and detectable dihydropyrrolizines in both species dissected, while abdominal contents usually had low PA in non-reproductive (winter dry season) individuals. The variation in total PA between adults in a given population (males as crosses on upper line, females as circles on lower in each graph) was random or polymodal, indicating a wide 'palatability spectrum'[7]. Individuals near the lower end, usually very young, would be true 'automimics' in the population and should be readily eaten by *Nephila* (Fig. 2). The patterns suggest opportunistic accumulation of alkaloid by each individual as various sources become available to the population, up to a maximum of over 13% of dry weight (at least in *Napeogenes*, *Ithomia* and *Pteronymia*; *Scada* males captured near *Eupatorium* flowers averaged over 13% and one showed over 20%), or 5.8 mg (in a male *Melinaea mneme*). Recently emerged adults (laboratory-reared or field-captured) showed no PA; a highly positive correlation was found in males, but not in females, between PA content and estimated age. Typical spreads, concentrations and male–female patterns in PA storage are shown by *Ithomia*. A few genera (*Tithorea*, *Placidula*, *Callithomia*, *Heterosais*, and especially *Methona*) show low accumulation in adults. *Tithorea*, *Aeria*, *Mechanitis*, *Rhodussa* and *Hypothyris* may show greater average storage of PAs in females than in males in some populations, but highest concentrations are always found in reproductively active males. Males attracted to baits (PA sources) and females attracted to displaying males showed low concentrations, suggesting an 'appetite effect'. In a notable case of a mating pair of *Melinaea menophilus*, the male was about to transfer 1.7 of his 3.6 mg of PA in a spermatophore (a small sac with less than 10% of his dry weight) to the virgin female who contained no PA. Other courting or mating males of seven genera analysed showed similar channelling of PA into spermatophores (up to 23% of dry weight or 50 times the concentration found in the rest of the organism), supporting a suggestion by L. Gilbert that females, rarely found on PA sources, get most of their alkaloid from males during mating. As they mate opportunistically and frequently, and place much of their PA on the eggs they lay, their content does not correspond so well with age.

Many pharmacological, physiological and ecological activities of dehydropyrrolizidine alkaloids (PAs) are known[15]. Danaine butterflies (sister-group to the Ithomiinae[16]) and arctiid moths sequester PAs from larval foodplants, or as adults obtain them from the same sources as do the Ithomiinae, using them as physiological regulators, pheromone precursors and defence substances[12,13,17–20]. In Danainae that store cardenolides erratically from larval food[21,22] PAs have often been suggested as alternate predator–defence compounds[13,21–24]. In *Utetheisa* (Arctiinae), larval-sequestered PA diesters protect adults against *Nephila clavipes*[18]. Some male Ithomiinae were suggested to use adult-sequestered PAs as hairpencil pheromone (Fig. 1*m*) precursors[12–14], but these butterflies have usually been assumed to sequester Solanaceae toxins for defence[1].

Methanol–water extracts, prepared by rapid vigorous homogenization of fresh leaves or flowers of larval and adult foodplants and fresh butterflies, were separated into medium-polarity neutral, alkaloidal and highly polar fractions, containing different classes of secondary compounds (Fig. 1). Further extraction with ethyl acetate gave an apolar fraction. Extracts and fractions were characterized by chromatography and ^{1}H-NMR spectra, and bioassayed with large populations of *Nephila* in ithomiine 'pockets'[2,3]. A small amount (corresponding to that extracted from 0.5–1.5 butterflies, 0.5 g fresh leaf of larval foodplant or 0.05 g fresh leaf or flowers of adult food), in aqueous solution or suspension, was applied with a soft brush onto the

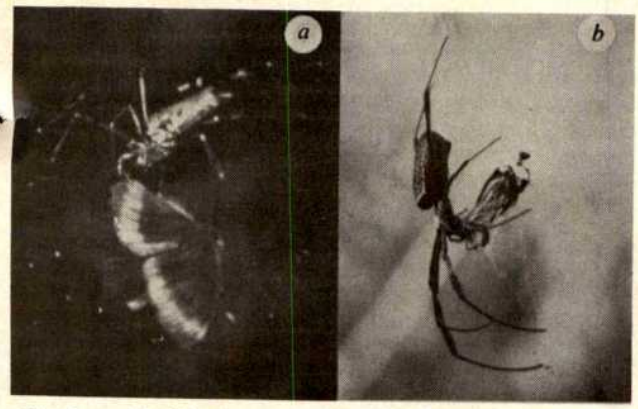


Fig. 3 *a, Nephila clavipes* cutting out a *Biblis hyperia* treated externally with the total MeOH–H_2O extract of one *Mechanitis lysimnia* adult. *b, Nephila* eating a freshly emerged *Pseudoscada erruca* adult, reared from the egg on *Cestrum laevigatum.*

body and wing bases of the common nymphaline *Biblis hyperia.* This was thrown into the web of a mature, non-satiated *Nephila*, which either readily ate the *Biblis* or cut it out (Fig. 3).

All *Biblis* treated with Solanaceae leaf extracts or fractions, or apolar or neutral fractions of Ithomiinae, like newly emerged adult Ithomiinae except *Aeria* (Apocynaceae-feeder), were accepted by *Nephila* (Table 1). In contrast, all total extracts and alkaloidal or polar fractions of field-captured Ithomiinae and their principal food sources caused *Nephila* to cut *Biblis* out just like intact wild Ithomiinae. All active extracts and fractions contained one or more PA monoesters (Fig. 1*e–i*), free or *N*-oxides. When freshly emerged adult Ithomiinae were fed pure PA *N*-oxide (0.3 mg) they became unpalatable to *Nephila* after about 1 h.

The distribution of PAs in Ithomiinae and food sources, measured by a modified Mattocks–Bingley assay[25,26], corresponds to their ecological and reproduction roles. The Eupatorieae, often pollinated by Ithomiinae[12], show highest concentrations in the flowerheads and especially nectaries, while the butterflies concentrate the alkaloids in the tegument (where the predators will immediately sense them), reproductive tissues and eggs (Fig. 2).

Most Ithomiinae found on PA sources are males[11,12]. In chemoassay, males of most genera show a broader range of PA content and higher extreme accumulation than females (Table 1, Fig. 3). Reproductively active males channel up to half their total PA into spermatophores that are transferred to females, who accept multiple matings and may obtain much PA in this fashion (Fig. 2).

Thus freshly emerged adult Ithomiinae, which unlike many Danainae and Arctiidae bear no accumulated toxins from larval foodplants, must find in their environment the PAs used in defence and reproduction. This necessity determines many chemical, physiological, behavioural, and morphological traits of the adult insects and of the plants they pollinate. Selection probably favours the obtaining of such essential compounds from a source stabilized by mutualism, rather than from the solanaceous larval foodplants, whose diversified chemical leaf constituents are constantly changing in response to the antagonistic setting and do not offer protection against *Nephila*, a major potential predator of Ithomiinae.

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Fibre type composition of single motor units during synapse elimination in neonatal rat soleus muscle

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Skeletal motor neurones innervate the specialized 'types' of fibres comprising most mammalian muscles in a characteristic fashion: each motor neurone forms a 'motor unit' by innervating a set of fibres all of the same type[1–4]. Because the type expression of adult muscle fibres is plastic and apparently controlled by their innervation, each motor neurone is thought to impose a common type differentiation on all the fibres in its motor unit[4–6]. However, the situation in developing muscles cannot be this simple. Muscle fibres in neonates receive synaptic input from several motor neurones and achieve the adult, single innervation only after a period of 'synapse elimination'[7,8]. Despite this polyneuronal innervation, differentiated fibre types are present in neonatal muscles[9–14]. This means either that the motor neurones polyneuronally innervate fibres in a random fashion and type expression is not determined by innervation or that the polyneuronal innervation is ordered in such a way that each fibre could receive unambiguous instructions for type differentiation. We have investigated these possibilities here by determining the fibre type composition of motor units in neonatal rat soleus muscle. We find that even during the time of polyneuronal innervation each motor neurone confines its innervation to largely one of two fibre types present in the muscle. Therefore, some mechanism during early development segregates the synapses of two groups of soleus motor neurones onto two separate populations of soleus muscle fibres.

We examined the soleus of Wistar rats at two postnatal ages—at 8 days, when every fibre is innervated by at least two motor neurones, and at 16–17 days, by which time synapse elimination has resulted in virtually every fibre being singly innervated[8]. In agreement with previous work[9–11], we found two distinct fibre types in soleus cross-sections stained for myofibrillar ATPase activity (Fig. 1). At both ages muscles showed a mosaic of lightly and darkly stained fibres. Because these neonatal muscles contain a number of novel embryonic and neonatal myosin

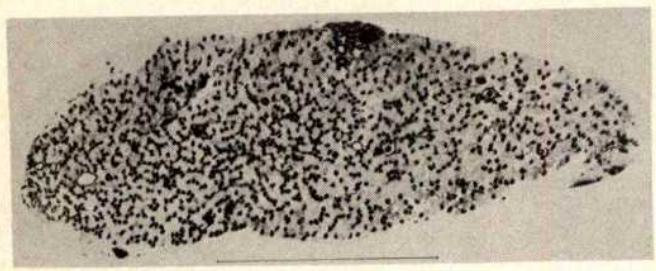


Fig. 1 Cross-section of a 16-day-old rat soleus muscle stained for myofibrillar ATPase activity after alkaline preincubation at *p*H 10.4 according to the method of Guth and Samaha[34]. Two muscle fibre types are visible, termed light and dark according to staining intensity. Scale bar, 1 mm.

isozymes[13,15–17], there is some uncertainty as to the exact adult fibre types to which each staining pattern corresponds. Although the light fibres presumably become type I fibres and the dark fibres type IIA fibres[9,10], we refer to these fibre types as 'light' and 'dark' based on staining intensity. Similar light/dark differentiation was seen at both ages (Figs 2*a*, *c*, 3*a*). Moreover, the total number of fibres and the proportion of each type appeared unchanged, despite the loss of over 50% of the synaptic connections in the muscle between 8 and 16–17 days.

To determine how single soleus motor neurones innervate these two fibre types, we used the glycogen depletion technique[18–20] to mark the fibres in a motor unit. The basis of this method is that stimulation of a single motor neurone activates the fibres comprising its motor unit and, in conditions of muscle anoxia, forces these fibres to utilize their glycogen stores. By staining muscle sections for glycogen, these fibres can be identified by their absent or weak coloration.

The procedures outlined by Kugelberg[19] were used for isolating and stimulating a single soleus motor axon in anaesthetized rats. Fibres in the motor units of stimulated axons were easily recognized in sections stained with the periodic acid-Schiff method[21] (Fig. 2*b*, *d*). Counts of depleted fibres showed that motor units at 8 days contained two to three times more fibres than those at 16–17 days (Fig. 3*b*), implying that the average muscle fibre at 8 days receives suprathreshold innervation from two or three motor neurones. These counts also offer some assurance that the depletion method is marking a large fraction of the fibres in units, as the present measurements are in reasonable agreement with estimates of motor unit size based on tension measurements (140 fibres at 16–17 days[22] and 340 fibres at 8 days[8]). Thus, glycogen depletion reaffirms the polyneuronal innervation of neonatal muscles and the decrease in motor unit size accompanying synapse elimination[8,23,24]. This decrease in unit size occurs as each motor neurone reduces the number of fibres it innervates, presumably as a result of competition among the terminals at each end plate[8].

The fibre type of each depleted fibre was determined by identifying the same fibres in two adjacent muscle sections, one stained for glycogen and the other for ATPase activity (Fig. 2). By examining complete cross-sections in this fashion, the fibre type composition of motor units was determined (Fig. 4). The striking finding is that motor units at both ages were largely homogeneous. Given the proportions of light and dark fibres present in soleus, a random innervation would be expected to yield units containing a mixture of about 55% light and 45% dark fibres. However, even at 8 days units consisted of at least 72% of one fibre type, and three of the five units examined were >90% homogeneous (Fig. 4). As motor neurones primarily innervate either only dark or only light fibres at day 8, most of the polyneuronal innervation of dark (or light) fibres must come from neurones which form motor units of the dark (or light) type. Furthermore, because of this predominance of synapses

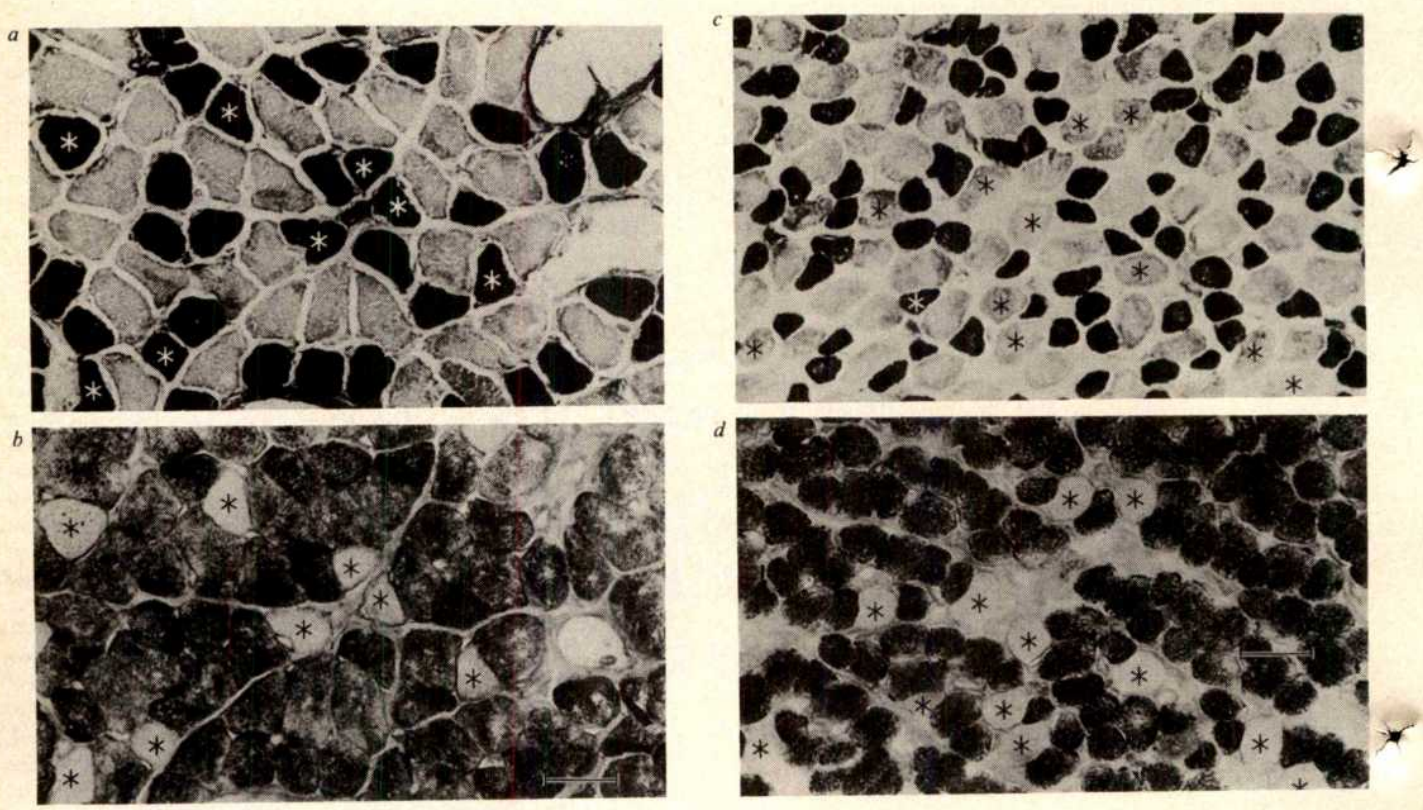
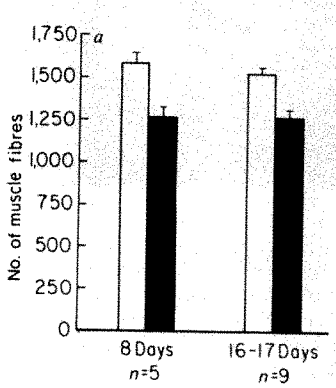


Fig. 2 Muscle cross-sections stained for myofibrillar ATPase activity after alkaline preincubation (*a*, *c*) and for glycogen (*b*, *d*). Adjacent sections stained by each method are shown for two muscles following stimulation of a single soleus motor axon. *a*, *b*, Portion of a motor unit from a 16-day-old rat. *c*, *d*, Portion of a motor unit from an 8-day-old rat. Asterisks mark glycogen-depleted fibres and the corresponding fibres stained for ATPase. Scale bars, 25 μm.

placed on 'appropriate' types of fibres, most of the synapse loss between 8 and 16–17 days occurs from appropriate as opposed to inappropriate fibre types.

The most intriguing finding is the segregation of innervation by two groups of soleus motor neurones. One obvious explanation of this segregation is a selective synapse formation between predetermined types of neurones and muscle fibres. Indeed, this type of selectivity has been previously proposed for other neuromuscular systems[25,26], and certain recent findings support the existence of some type of predetermination of fibre type[12,27–29]. Other observations, however, militate against such selectivity. First, reinnervation of adult fibre types is nonspecific and fibres mismatched with their innervation are gradually transformed in fibre type[30]. Second, the differentiation of one type of fibre (slow, type I) seems to be neurally induced, as this type is absent in muscles denervated at birth[11,27,31]. Moreover, there are certainly alternative explanations of the segregation which do not require a predetermination of muscle fibre types. In fact, the observed segregation of innervation is the result required if fibre types were to be dictated by instructions from innervating axons during polyneuronal innervation.

How might the segregation occur in the absence of predetermined fibre types? Two preexisting types of motor neurones might segregate their synapses onto separate naive muscle fibres as a result of an interaction among nerve terminals either during synaptogenesis or during a subsequent period of synapse elimination preceding 8 days. An alternative suggestion made by Rubinstein and Kelly[12] involves the timing of synaptogenesis and myogenesis: if two groups of motor neurones arrive in the muscle at different times, coinciding with separate waves of myogenesis, these neurones could be constrained to innervate different sets of muscle fibres polyneuronally. Exactly how muscle innervation becomes segregated is unknown, but it is now evident that the generation of homogeneous motor units does not await the single innervation of muscle fibres.



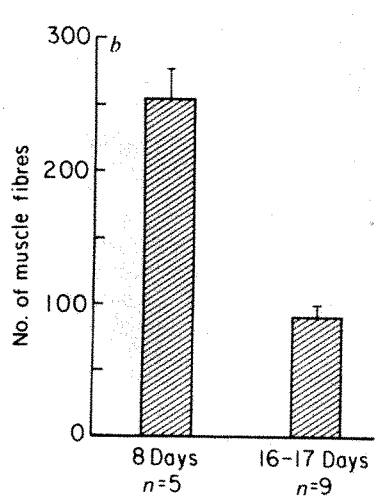


Fig. 3 Changes in muscle fibre type composition and motor unit size between 8 and 16–17 days. *a*, Number of dark (filled bars) and light (open bars) fibres found in muscles of each age. *b*, Numbers of muscle fibres comprising single motor units as determined by glycogen depletion. Vertical lines represent s.e.m.

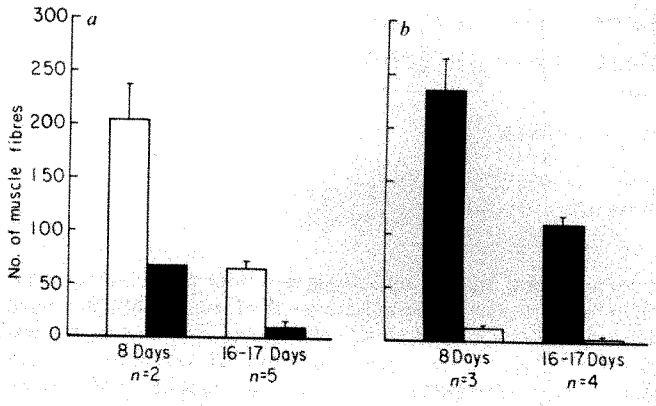


Fig. 4 Fibre type composition of motor units in soleus muscles at 8 and 16–17 days. Numbers of fibres of the dark (filled bars) and light (open bars) types are shown for motor units at each age. *a*, Units composed predominantly of light fibres. *b*, Units composed predominantly of dark fibres. Vertical lines represent s.e.m. (No vertical line appears for two units in the 8-day muscles in *a* which each contained 66 dark fibres.)

We acknowledge that H. Gordon and D. Van Essen have reached similar conclusions to those reported here by their study of the contractile properties of motor units in neonatal rabbit soleus muscle (ref. 32 and work in preparation).

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Is stepwise sarcomere shortening an artefact?—a response

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Like any other signals, optical signals from muscle fibres inevitably contain noise. The noise here is of predictable character. Because the structure of muscle is periodic, or almost so, translational movement of an illuminated fibre will inevitably give rise to a periodic noise fluctuation as striations pass across the optical field. The frequency of this fluctuation should be linearly related to the speed at which the fibre translates. By mistaking this noise component for the signal, Altringham and colleagues[1] have amply succeeded in confirming this expected relationship, but they have left unanswered the question of the source and nature of stepwise shortening. I show here that: (1) by having obtained records in conditions in which the noise dominated, Altringham *et al.* have inadvertently confused the steps with the translation-induced fluctuation; and (2) when records are obtained using a method designed specifically to circumvent the effects of translation, the steps still remain in evidence.

Consider first the authors' Fig. 1 (ref. 1). To evaluate their method of analysis, consider, for example, the record labelled '2.72 μm' in Fig. 1*b*. This record is not unlike many we have obtained and published[2,3]. How many steps are there? I identify one pause (of moderate 'quality'), and hence two steps. The authors, however, have mistakenly confused the high-frequency fluctuations superimposed on the trace for the steps. Consequently, they counted 47 instead of 2. Records such as these gave the mid- to high-frequency points on their Fig. 1*a*, drawing the authors towards the inappropriate conclusion that the step frequency was linearly related to translation velocity. Instead, what they have shown is that it is the fluctuation that is linearly related to translation velocity. Had the authors plotted step frequency instead of fluctuation frequency, the resulting scatter diagram would have led them to an opposite conclusion to the one they drew.

The authors' Fig. 2 (ref. 1) illustrates the results obtained with the phase-locked loop (PLL, striation imaging) method. Consider the first (18-4-83) of the two experiments. As waveforms for this day's experiment were not shown by the authors, I have reproduced representative samples here in Fig. 1*a*, and show comparable records taken from our recent experiments in Fig. 1*b*. Note that the noise levels are 20–50 nm and 2–3 nm respectively. A step size of 10–20 nm, characteristic of our records, would therefore be buried in their noise. The authors, once again, appear to have studied a fluctuation.

Such fluctuations have, of course, been of concern to us. The PLL method inherently generates translation-induced fluctuations[5], but we have demonstrated that with signals of reasonable quality, their amplitude remains 2 nm or less[4]. However, we have also obtained our share of records as noisy as those in Fig. 1*a* when one of a number of conditions had not been carefully controlled. Inevitably, the translation-induced fluctuation becomes the dominant feature of such records and masks the actual sarcomere shortening signal. While we ordinarily discard such records, Altringham *et al.*[1] have capitalized on them and have elegantly confirmed that the translation-induced oscillations correlate well with translation ($r = 0.999$).

In the second of the two PLL experiments, illustrated in the authors' Fig. 2*b*, Altringham *et al.* appear to be dealing with genuine sarcomere-length signals. However, their protocol ensures that the linear relationship will be found. In this second experiment, observation was restricted to a single region, while the moving end of the fibre was released at each of various speeds. Increasing the speed of release brings with it two (correlated) effects: it increases the local sarcomere shortening velocity, causing the steps to occur more frequently, and it also increases the local translation velocity. Thus, a linear relationship between step frequency and translation velocity is an inevitable consequence of their protocol. Further, since the translation velocity will increase from the fixed to the moving end, the slope of this linear relationship is not unique; it will depend on the choice of region to be studied. The particular value of slope found in this experiment is therefore without significance.

Finally, with regard to the authors' Fig. 3, we previously published essentially identical experiments except with 1,000 times higher time resolution. We showed that in regions which did not translate excessively, the first-order profile remained steady during the pause[3]. This convinced us that the pause represented a truly stationary period. Our conclusion did not imply that changes in first-order profile could not accompany the pause. Clearly, in the experiments of Altringham *et al.*, where substantial translation was an essential element in the protocol, and where only those diffraction patterns with 'less than

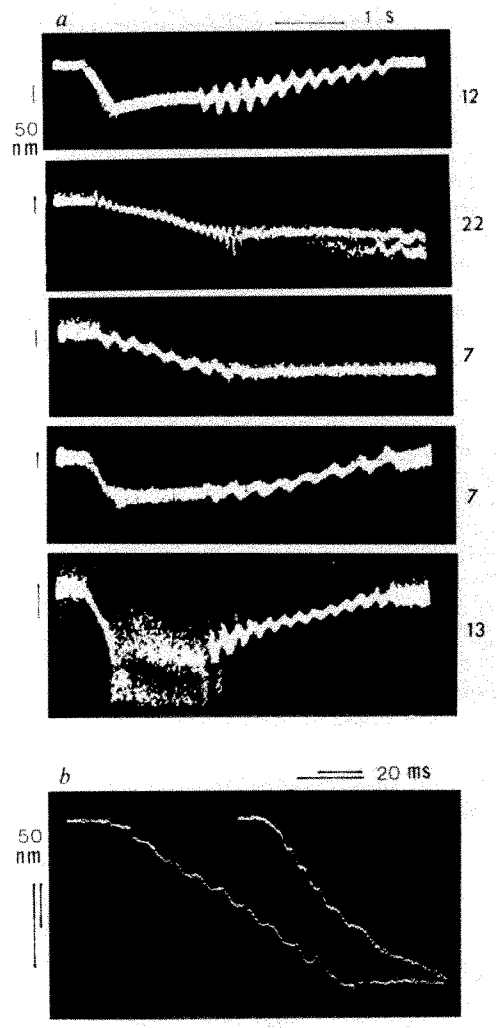


Fig. 1 Time course of sarcomere shortening measured with the phase-locked loop (PLL) imaging method. The five traces in *a* were obtained in the experiment of 18-4-83 and were kindly provided by Altringham *et al.*[1]. (These are not worst-case records; peak-to-peak noise averaged among all 20 records was 32.5 nm, in the range of the five shown here.) Time bar calibration refers to all but the third panel, which is 200 ms. The number of oscillations counted by Altringham *et al.* is given at the right of each panel. The traces in *b* are representative of those obtained in experiments on unstimulated (left) and stimulated (right) frog toe fibres in this laboratory. They illustrate the character and size of the steps that we have measured[4,15]. Note that steps of this size would probably be submerged in the noise of the upper set of records. Also note the vast differences of time scale between *a* and *b*.

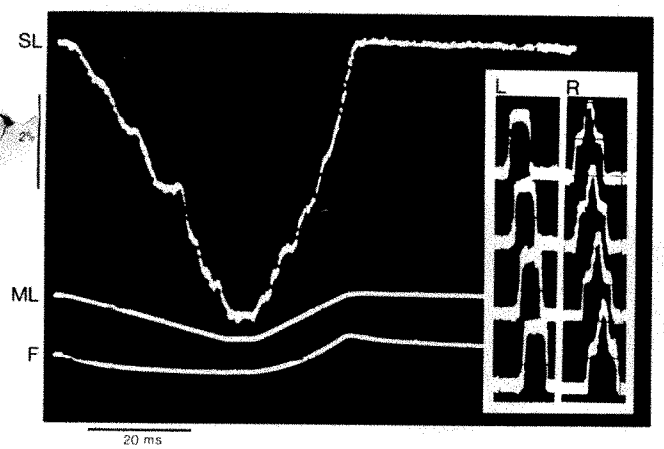


Fig. 2 Time course of segment shortening in an unstimulated single fibre of frog tibialis anterior muscle[6]. SL, segment length; ML, muscle length; F, force. Initial segment length was 515 μm. The segment is defined by the spacing between two hairs, nominally 10 μm in diameter and 80 μm in length, mounted on the upper surface of the fibre, perpendicular to its long axis. The image of the section of fibre containing the two hairs was split such that one hair was projected onto one photodiode array, while the other was projected onto a second array. A processing circuit computed the area under the image of each marker, from which the respective median locations were computed. The difference between median locations gave the segment length. Thus, the method is insensitive to translation. Of 17 fibres that gave usable segment length signals, 11 showed distinct, repeatable steps. The two inset panels show the intensity profiles of the left (L) and right (R) markers at four instants (top to bottom) spanning the longest shortening pause. They demonstrate that spurious fluctuations of intensity profile of either marker were not present, and therefore could not have given rise to an artefactual pause. Edman *et al.*[8], using a similar method, also observed pauses in some records of segment length change (see their Fig. 8).

optimum quality' could be used, the detection of such changes is not at all surprising.

Thus, by using (1) noisy PLL signals, (2) weak diffraction patterns and (3) protocols in which translation was emphasized, Altringham and colleagues have set their attention on signals dominated by noise. In so doing, they have obtained clear proof that the noise arises out of fibre translation. It is unfortunate that the superficial resemblance between the translation-induced artefact and the stepwise shortening signal has given rise to this confusion. In fact, signal and noise are quite distinct. In the optimized conditions that we have been trying to realize since our early experiments (the reverse of (1)–(3) above), patterns such as those of Fig. 1*b* have begun to emerge consistently and serve to define the phenomenon. In such low-noise records, the shortening waveform approaches a true staircase, with clear pauses and regular cascades of steps of predictably distinct size[4,15]. By contrast, the fluctuations shown in the authors' waveforms are of broadly variable size and character. The resemblance is thus only superficial.

Nevertheless, in light of the potential for confusion, we have designed a simple experiment to determine whether steps could still be observed in conditions in which the effects of translation were circumvented[6]. Using a segment-length detection method similar in principle to that developed by Gordon, Huxley and Julian[7], we have observed stepwise shortening regularly in both stimulated and unstimulated fibres (Fig. 2). Similar waveforms have been found with other segment-length methods in three laboratories (refs 8, 9 and T. Tameyasu and H. Sugi, in preparation). We conclude, therefore, that steps arise independently of translation.

The phenomenon of stepwise shortening is not easily reconciled with commonly accepted views of contraction, as the authors state[1]. This is perhaps why the challenge by Altringham *et al.* is not unique; it represents the fourth in a series, each laying claim to identification of the long-sought artefact[1,10–12]. Yet the phenomenon remains robust. It has now been observed in a variety of laboratories using four classes of methodology: laser diffraction[2,3,10,13], on line imaging of the striations[4,15], high-speed cinemicrography (ref. 14 and H. Sugi and M. Toride, in preparation), and various segment-length methods (refs 6, 8, 9 and T. Tameyasu, T. Yoyoki and H. Sugi, in preparation). Although some unsuspected artefact may yet remain, I know of no other phenomenon that has been confirmed by as many distinct methods as this.

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Sir Andrew Huxley comments

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POLLACK says that the fluctuations illustrated in Fig. 2*b* of the article by Altringham *et al.* 'appear to be genuine sarcomere-length signals'. However, the fact pointed out by Altringham *et al.* that 'one oscillatory cycle corresponds to the translation of one sarcomere past a given point in the optical field' makes it practically certain that in this case the fluctuation is an instrumental artefact. The existence of 'noise' fluctuations of this kind is admitted by Pollack. Its origin in experiments using the PLL device is clearly seen in Fig. 1 of Pollack's ref. 4: the crude frequency signal fluctuates at one cycle per sarcomere in the image, and this fluctuation will be eliminated only if the number of sarcomeres m over which the signal is averaged is an integer (n). m depends on sarcomere length, and when $m = n + 1/2$, the peak-to-peak fluctuation in the average as the image moves across the field is $A/\pi m$, where A is the peak-to-peak amplitude fluctuation in the signal being averaged. With the values given in Pollack's ref. 4, this comes to 3.6 nm, which is close to the amplitude of the deviations from a smooth curve in Pollack's Fig. 1*b* and in Fig. 2*b* of Altringham *et al.*, both of which are taken by Pollack to represent genuine changes of sarcomere length.

These reasons for believing those fluctuations to be artefactual are not upset by Pollack's claim that 'a linear relationship between step frequency and translation velocity is an inevitable consequence of their [Altringham *et al.*'s] protocol': true, but the protocol does not ensure one cycle per sarcomere, which would be an unlikely coincidence unless the fluctuation is an instrumental artefact. Pollack's suggestion that the slope of the relationship 'will depend on the choice of region to be studied' is contradicted by the observation of Altringham *et al.* that 'the frequency of the oscillations appears to be determined only by the translation velocity of the fibre at the point observed, and not by the rate of change of sarcomere length'.

I conclude that Altringham *et al.* are right in claiming that the rather regular fluctuations often seen with the PLL method

are an artefact. The 'one pause' claimed as real by Pollack lasts for about 10 sarcomeres of fibre displacement, and may therefore be simply the consequence of a difference of sarcomere length between two regions in the fibre.

Details of the 'simple method' referred to by Pollack, with which his Fig. 2 was obtained, have not yet been published (his ref. 6 is 'in the press'), so there is no way of telling to what errors it is subject. There are many possible sources of error; for example, sideways movement of the fibre if the markers are not perpendicular to the fibre axis, or vertical movement of the fibre if the light beams used for following the two markers are not parallel to one another.

Transposition bursts in genetically unstable *Drosophila melanogaster*

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A genetically unstable strain of *Drosophila melanogaster*, ct^{MR2}, which appeared in the course of hybrid dysgenesis[1], was found to contain an unstable mutation in the *cut* locus, resulting from an insertion of the mobile element *mdg4* (ref. 2). Multiple genetic events, probably resulting from transposition, were found in this strain and its derivatives[3]. We have analysed the localization of various mobile elements within the X-chromosome of the ct^{MR2} strain and its derivatives. We report here a dramatic change in the localization of mobile dispersed genetic elements *mdg1, mdg2, mdg3,* and *copia*, FB- and P-elements within the X chromosome. Up to 20 independent transposition events were detected. As the localization of mobile elements within ct^{MR2} and within each substrain is invariant, we conclude that all transpositions occurred simultaneously in one and the same germ cell. Such 'transposition bursts' may have an important role in evolution.

The genetically unstable strain ct^{MR2} gives rise to several stable and unstable ct^+ reversions and novel *ct* mutations[1,3]. These are frequently accompanied by other unstable mutations. All *ct* mutations including *ct* lethals contained *mdg4* in the 7B region of the X chromosome[2].

Using *in situ* hybridization with polytene chromosomes, we have analysed the localization of different mobile elements in the X chromosome of the ct^{MR2} strain (Figs 1–3). Larvae were examined at various times, in particular almost immediately after obtaining the ct^{MR2} strain and 2 years later. In all cases, the localization of mobile elements was exactly the same (the number of individual larvae analysed totalled 20). Thus, different mobile elements including *mdg* (*copia*-like elements), P-elements and FB-elements, have rather stable localizations. However, the analysis of novel substrains derived from ct^{MR2}, as a result of transposition event(s) in *cut* or other loci, reveals a dramatic change in the localization of mobile elements (Figs 1, 2). The two most extensively studied examples are shown in Fig. 1. The substrains analysed were ct^+ revertants which occurred simultaneously with unstable mutations at the *singed* or *white* loci. All mobile elements showed evidence of transposition, with their appearance at new, and disappearance from old, sites. In the case of $ct^{MR2} \rightarrow ct^+sn^{MR2}$, 20 simultaneous transposition events were counted, and 12 were found for the $ct^{MR2} \rightarrow w^{MR1}$ *ct* mutation.

It is important to stress that all individuals of the new derivative strains possess the same distribution of mobile elements—this means that all transposition events occurred simultaneously in the same germ cell. Had different transpositions occurred at different stages, there would be no homogeneity within the strains.

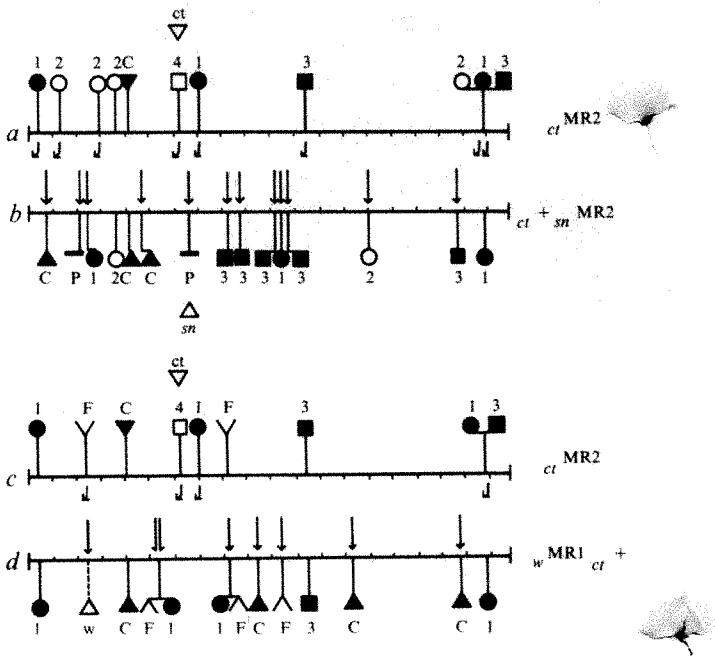


Fig. 1. Schematic presentation of the results obtained for *in situ* hybridization of ct^{MR2}, ct^+sn^{MR2} and $w^{MR1}ct^+$ X chromosomes with different probes. *a, c*, Distribution of hybridization sites along the ct^{MR2} X chromosome; *b*, that of the ct^+sn^{MR2} X chromosome; *d*, that of the $w^{MR1}ct^+$ X chromosome. In *a, b* the hybridization was performed with *mdg1* (1), *mdg2* (2), *mdg3* (3), *mdg4* (4), *copia* (C) and P-element (P); in *c, d* with *mdg1, mdg3, mdg4, copia,* P-element and FB-element (F). Triangles indicate the location of mutations characteristic of the strains analysed. Arrows show transposition events: the loss of mobile elements (⇃) and their insertion (↓). Here and in the other figures, the 20 divisions on the line representing the X chromosome indicate the 20 major divisions of the chromosome on a Bridges map, the centromere being to the right. The strain ct^{MR2} remained unstable for 1.5 years, thereafter the transposition rate decreased, but later on increased. The strain was homozygous with respect to the X chromosome. ct^+ reversions and novel unstable mutations were observed in the males of the ct^{MR2} strain. To obtain the corresponding substrains, we used one of two methods. (1) The mutants were crossed with females bearing attached X chromosomes. Further, only the X chromosomes of male larvae were analysed. (2) The mutants were crossed with females possessing an X chromosome with a multiple inversion *FM4*, y^{31d}, sc^8dm *B* that excluded crossing-over. In this case, novel substrains homozygous with respect to the X chromosome (originating from ct^{MR2} X chromosome which has avoided crossing-over) were obtained. This made it possible to analyse both male and female X-chromosomes.

Figure 2 shows two other examples of multiple transpositions in the X chromosome of ct^{MR2} derivatives. In these cases, the ct^{MR2} mutation did not change, but a new unstable mutation occurred at the *garnet* (*g*) or *Bar* (*B*) locus. As the ct^{MR2} mutation was conserved, the *mdg4* remained in the 7B region. However, in both cases, several transpositions were found for other *mdg* elements and for FB-elements. Thus, the loss of *mdg4* from the 7B locus is not necessary for the induction of transposition. It should be pointed out that, in $ct^{MR2}g^{MR1}$, a *g* mutation seems to be induced by a *copia* insertion, as g^+ reversion always coincides (four cases were tested) with a loss of *copia* from the 12BC region.

We have also analysed several other ct^{MR2} derivatives for localization of *mdg1* or *copia* (Fig. 3). It is clear that almost always two to four transposition events occur in the X chromosome.

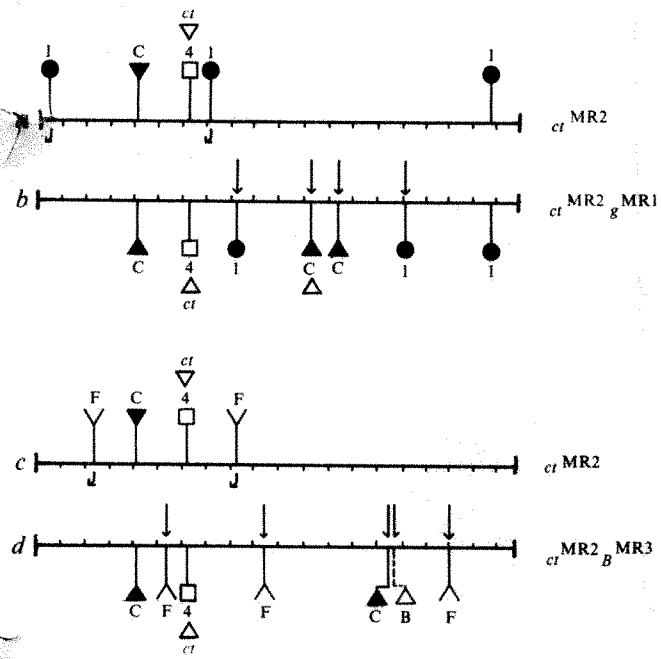


Fig. 2 Schematic presentation of the results obtained for *in situ* hybridization of ct^{MR2}, $ct^{MR2}g^{MR1}$ and $ct^{MR2}B^{MR3}$ X chromosomes with different probes. *a*, *c*, Distribution of hybridization sites along the ct^{MR2} X chromosome; *b*, that of the $ct^{MR2}g^{MR1}$ X chromosome; *d*, that of the $ct^{MR2}B^{MR3}$ X chromosome. In *a*, *b* the hybridization was performed with *mdg1*, *mdg4* and *copia*; in *c*, *d* with *mdg1*, *mdg4*, *copia* and FB-element. Designations are the same as in Fig. 1. The method used to isolate the substrain was as described in Fig. 1 legend.

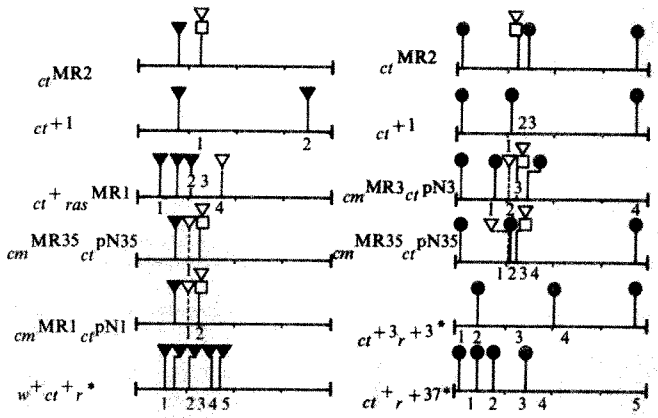


Fig. 3 Results of *in situ* hybridization of the X chromosomes from different ct^{MR2} derivatives with *mdg1* and *copia*. Most of the substrains appeared as the result of a mutation in ct^{MR2}. Those marked by asterisks were obtained from the $ct^{MR2}r^{MR1}$ substrain (which, in turn, originated from ct^{MR2}). The numbers represent the number of the transposition event. Designations are the same as in Fig. 1. Triangles indicate the locations of mutations in the individual strains.

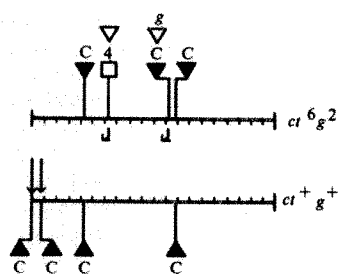


Fig. 4 Comparison of the distribution of *mdg4* and *copia* in the stable and destabilized ct^6g^2 strain and the ct^+g^+ double revertant obtained from ct^6g^2. Designations are the same as in Fig. 1.

The ct^{MR2} strain is not the only strain in which multiple transpositions can be observed in few germ cells. The ct^6g^2 strain (from the Mid-America *Drosophila* stock centre, Bowling Green State University), destabilized by a cross with the P-factor-containing strain *MRh12/Cy* (ref. 4), is another example. In the destabilized strain, a reversion to the g^+ and ct^+ type occurs at a rate of $\sim 10^{-3}$. In half of the cases, a double ct^+g^+ reversion takes place. The X chromosomes from stable and destabilized ct^6g^2 strains contain four *copia* sites (Fig. 4); different individuals possess the same distribution of *copia* sites. In the offspring of one ct^+g^+ revertant, however, one *copia* site was lost and two new sites were accepted (Fig. 4). Thus, three transposition events concerning only one *mdg* element occurred in the same germ cell.

We conclude, therefore, that in *Drosophila melanogaster* strains destabilized by hybrid dysgenesis, transpositions occur in only a few germ cells, but are numerous in these particular cells. 'Transposition bursts' have occurred in these cells.

Transposition bursts involve at least three major types of mobile elements: (1) mobile dispersed genes (*mdg*) or *copia*-like sequences[5,6]; (2) foldback sequences[7]; and (3) P-elements[8]. Hence, all of these may be mobilized by a similar mechanism.

The total number of transposition events should be at least an order of magnitude greater than that determined in our experiments. We analysed the locations of only six different families of mobile elements and in only one chromosome. Even then, up to 20 simultaneous transposition events were detected; thus, the total number may be several hundreds.

Transposition bursts seem to occur only rarely in germ cells ($\sim 10^{-3}$) but this value may be an underestimate, as we expect that the insertion of a mobile element would occasionally be lethal and the respective germ cells or embryos would not survive. In fact, the homozygous ct^{MR2} strain is characterized by a high rate of appearance of sex-linked lethals[1].

The question arises as to which factors control multiple transposition. In our system (ct^{MR2}), P-factor seems to be an important component[2,3]. However, although the P-factor is present in all cells of a given organism, transpositions occur in only a few of them. Probably some other trigger mechanism exists.

The saltatory character of transposition may have a significant role in evolution. It is well known that the insertion of mobile elements is an important factor in spontaneous mutagenesis[9]. Therefore, transposition bursts should lead to simultaneous multiple mutagenesis, creating an organism that differs in many feautres from its parents. One of the problems in theories of evolution is how to explain the appearance of new complex properties requiring several independent genetic changes, each of which is not selectively advantageous—transposition bursts may provide the answer. Multiple mutations could occasionally create such complex features, which may then be retained by selective pressure.

In our system, transpositions occur at a premeiotic stage and, as a result, novel mutants sometimes appear in clusters[1]. This may help changed genomes to survive in further generations.

Finally, transposition events can sometimes lead to gross chromosomal rearrangements. Thus transposition bursts may also assist in creating the reproductive isolation of a new substrain, the process having a key role in speciation.

We thank Drs D. Finnegan, G. Rubin and S. Potter for the clones containing *copia*, P-factor and FB-elements, Dr M. M. Green for the *MRh12/Cy* stock, and Dr M. Ashburner for critical reading of the manuscript.

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Control of tubulin gene expression in the parasitic protozoan *Leishmania enriettii*

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The life cycle of parasitic protozoa of the genus *Leishmania* consists of two morphologically distinct forms[1]: (1) amastigotes, the form of the parasite that resides inside macrophages of the mammalian host, which are non-motile and possess only a residual flagellum; and (2) promastigotes, the extracellular forms that live in the gut of the sandfly vector, which are highly motile and possess a single prominent flagellum. During the developmental transformation of amastigotes to promastigotes, the biosynthesis of α- and β-tubulin proteins is dramatically increased[2], presumably to provide sufficient tubulin for synthesis and maintenance of the flagellum. We show here that the level of α- and β-tubulin mRNA in *Leishmania enrietti* promastigotes is significantly greater than that in amastigotes, as determined by both Northern blot analysis and by *in vitro* translation of cellular RNA. These results show that the regulated expression of the tubulin genes is controlled at the level of mRNA accumulation in *L. enriettii*, by contrast with *Leishmania mexicana*, in which the control of gene expression has been reported to be at the level of translation.

The α- and β-tubulins are the major developmentally regulated gene products during transformation of *Leishmania* amastigotes to promastigotes. The α- and β-tubulin mRNAs are the most abundant messages present in the promastigote stage of the life cycle[3]. Biosynthesis of α- and β-tubulin occurs at a low level in amastigotes but is dramatically increased during their transformation to promastigotes[2]. On the basis of *in vitro* translation experiments, Wallach *et al.*[3] have suggested that control of tubulin biosynthesis may occur at the level of translation. In a recent extension of their work on *L. mexicana*, Fong *et al.*[18] have used heterologous tubulin probes and have found similar levels of tubulin mRNA in promastigotes and amastigotes. In contrast, we find, using homologous cloned copies[4,5] of the α- and β-tubulin genes, significantly more α- and β-tubulin mRNA in promastigotes than in amastigotes of *L. enriettii*.

L. enriettii promastigotes and amastigotes were grown as described in Fig. 1 legend and total nucleic acid extracted. Equal amounts of total nucleic acid from promastigotes and amastigotes were resolved on denaturing agarose gels, transferred to nitrocellulose and probed sequentially with the α- and β-tubulin genomic clones. The filters were re-probed with a cloned ribosomal gene as an internal control. Figure 1 and Table 1 show that the promastigotes contain a minimum of 4–5-fold more α- and β-tubulin mRNA than the amastigotes. Similar results were obtained when the amount of α- and β-tubulin mRNA was measured by *in vitro* translation in the rabbit reticulocyte system (see Table 1). Based on these results, we conclude that the control of α- and β-tubulin synthesis occurs primarily at the level of mRNA accumulation.

The control of tubulin gene expression has been studied in several other systems and has, in general, been found to occur

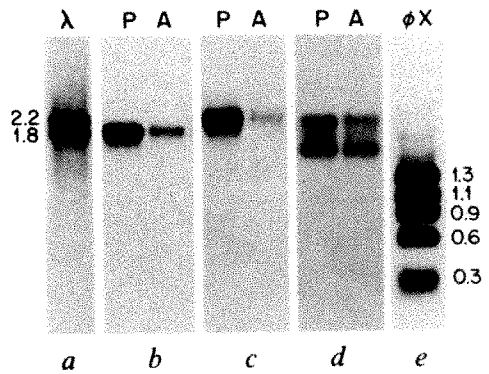


Fig. 1 Estimation of α- and β-tubulin RNA levels in promastigotes and amastigotes by Northern blot analysis. Five micrograms of total nucleic acid isolated by phenol–chloroform extraction from promastigotes (P) or amastigotes (A) of *Leishmania enriettii* were separated on a 1% agarose–formaldehyde gel[12] and blotted onto nitrocellulose. The same filter was sequentially hybridized[4] with nick-translated[15] α-tubulin clone pLTI (ref. 5) (*b*), β-tubulin clone pLEβ3 (S.M.L. and D.F.W., in preparation) (*c*) and with a ribosomal DNA clone (*d*) from *Plasmodium lophurae*[16]. Lane *a* contains *Hin*dIII fragments of λ DNA and lane *e* contains *Hae*III fragments of ΦX174 DNA hybridized to homologous probes. The numbers indicate the size of these molecular weight markers in kilobase pairs. The results in lanes *b* and *c* indicate that the α- and β-tubulin mRNAs in *L. enriettii* are both ~2 kilobases long. In addition, a heavier exposure of the autoradiogram in *c* (not shown) reveals lightly hybridizing bands both above and below the main band.

Methods: Promastigotes were grown to a density of $\sim 2 \times 10^7$ organisms ml^{-1} in Schneider's medium containing 10% heat-inactivated fetal calf serum. Amastigotes were grown by subcutaneous injection of either promastigotes or amastigotes into the noses of male Hartley guinea pigs. Lesions were allowed to develop for about 5 weeks, and parasites were isolated and purified by Percoll gradient centrifugation as described elsewhere[17]. This method yielded viable amastigotes at ~95% purity. RNA was prepared by suspending about 10^{10} parasites in 10 ml of 50 mM HEPES *p*H 7.5, 50 mM KCl, 10 mM EDTA, then lysing the cells with 0.1% Sarkosyl, and immediately extracting three times with 2 vol. phenol–chloroform and once with 2 vol. chloroform.

Table 1 Quantitation of tubulin RNA in promastigotes and amastigotes

	Promastigote/amastigote ratio
α-Tubulin	
Northern blots	
Expt 1	3.5:1
Expt 2	3.9:1
In vitro translation	1.9:1
β-Tubulin	
Northern blots	
Expt 1	5.3:1
Expt 2	4.0:1
In vitro translation	3.7:1

Autoradiograms of the Northern blots in Fig. 1 were quantitated by densitometry. Duplicate lanes were run for each determination, the integrated areas under the peaks were averaged, and these averaged areas were then normalized using the area under the ribosomal RNA peaks. This normalization adjusts for any small differences in the amount of total RNA actually loaded onto promastigote or amastigote lanes. The amount of tubulin RNA measured for the amastigote lane was then arbitrarily assigned a value of 1.0, and the relative amount for promastigotes was calculated. Experiment 1 was performed with RNA isolated by phenol–chloroform extraction (see Fig. 1), while expt 2 was performed with RNA isolated by the guanidinium isothiocyanate procedure[12]. For *in vitro* translation, 2 μg of the RNA samples used in Fig. 1 were translated in the rabbit reticulocyte lysate system[13] (25 μl total volume), and the products were resolved on an 8.5% Laemmli gel[14]. The α- and β-tubulin translation products were quantitated by densitometry of the autoradiogram.

at the level of mRNA accumulation[6-11]. Specifically, flagellar regeneration in *Chlamydomonas rheinhardii* is accompanied by an increase in α- and β-tubulin mRNA levels as determined by *in vitro* translation and Northern blot analysis[6,7]. We find that this is also the case for the α- and β-tubulin genes of *L. enriettii*. This is in contrast to the *L. mexicana* system[3,18] in which similar amounts of tubulin mRNA are reported to be present in the two forms of the parasite. Our results, based both on the direct measurement of mRNA levels by Northern blot analysis using homologous probes and on the *in vitro* translation of mRNA in the rabbit reticulocyte system, clearly indicate that there is several-fold more α- and β-tubulin mRNA in *L. enriettii* promastigotes than in amastigotes. There are two possible explanations for the differences found in the two *Leishmania* systems: either the control of tubulin levels is different in the two species, or the results reflect a difference between the heterologous probes used in the *L. mexicana* experiments[18] and the homologous probes used in our experiments. Thus, the stage-specific regulation of tubulin expression in *L. enriettii* is controlled at the level of mRNA accumulation. Whether this control is exerted through regulation of transcription or through RNA processing or degradation remains to be determined.

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Purification, sequencing and synthesis of natriuretic and vasoactive rat atrial peptide

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Mammalian atria contain potent natriuretic and diuretic substances[1,2] which exist in high- and low-molecular-weight forms[2,3] and which appear to be associated with atrium-specific granules[4]. The natriuretic effect of atrial extract is largely accountable for by its renal haemodynamic effects[5-7]; atrial extracts also antagonize hormone- and non-hormone-induced contraction of the isolated rabbit aorta[8-10] and isolated rat kidney vasculature[6]. We have completely purified a low-molecular-weight natriuretic and vasoactive substance from rat atria and characterized it as a 24-amino acid peptide. Synthetic peptide, produced by solid-phase synthesis, mimics biological effects of crude atrial extract and purified peptide; its activity is enhanced by slow oxidation, suggesting a disulphide (Cys 4–Cys 20) configuration for the native peptide. If secreted into blood, this atrial natriuretic peptide ('auriculin B') could be a novel peptide hormone of considerable importance to renal and cardiovascular homeostasis.

Atrial natriuretic factor (ANF) was purified from an acetic acid extract of atria from 1,400 male Wistar rats by gel filtration and reverse-phase HPLC (Fig. 1). Fractions were assayed for natriuretic activity either in the intact rat[1] or in isolated perfused rat kidney[6,11], and for vasorelaxant activity in rabbit thoracic aortic rings[10] contracted with either angiotensin II or histamine. Two broad regions of natriuretic and vasorelaxant activities were found on Sephadex G-50, corresponding to molecular weights of >15,000 and <6,000 (Fig. 1*a*). Application of the low-molecular-weight material to a C_{18} column resulted in a broad active fraction which, upon re-chromatography, was resolved into three peaks of coincident vasorelaxant and natriuretic activity (Fig. 1*b*). One of these was further purified by re-application to the same column (Fig. 1*c*) followed by chromatography on a CN reverse-phase column, which resulted in the association of vasorelaxant activity with a single UV-absorbing peak (Fig. 1*d*).

Table 1 Natriuretic effect of synthetic auriculin in intact rats

Dose (μg per kg)	$\Delta\dot{V}$ (μl min^{-1})	$\Delta U_{Na}\dot{V}$ (μequiv min^{-1})	$\Delta U_K\dot{V}$ (μequiv min^{-1})
1.2 (4)	25.5 ± 9.7	2.5 ± 1.1	1.6 ± 0.2
2.4 (4)	41.3 ± 19.5	6.7 ± 4.1	4.4 ± 1.4
5.0 (4)	52.8 ± 6.5	9.1 ± 1.0	3.7 ± 0.5
7.2 (3)	112.0 ± 12.8	18.3 ± 0.5	3.1 ± 0.8

Synthetic peptide was administered as a bolus injection to rats (average weight 399 g; number of animals in parentheses) anaesthetized with Inactin (100 mg per kg) and maintained on a constant infusion of normal saline (2.2 ml h^{-1}). The change in each parameter was assessed by the difference between the average of three control periods (10 min each) and the first experimental period (maximum response). Data are expressed as the mean ± s.e. $\dot{V}$, urine flow rate; $U_{Na}\dot{V}$, urinary sodium excretion rate; $U_K\dot{V}$, urinary potassium excretion rate. Control values for the 15 animals were: $\dot{V}$, 10.3 ± 2.9 μl min^{-1}; $U_{Na}\dot{V}$, 0.93 ± 0.5 μequiv min^{-1}; and $U_K\dot{V}$, 1.6 ± 0.4 μequiv min^{-1}.

Automated microsequence analysis of the purified substance revealed that it was a 24-amino acid residue peptide rich in glycine, serine and arginine (Fig. 2). This peptide is smaller than the ANFs purified to apparent homogeneity by deBold and Flynn[12] and by Grammer *et al.*[13]. It also differs from the sequences reported recently by Currie *et al.*[14] in that it has an additional arginyl residue at the amino terminus.

Solid-phase synthesis of the peptide shown in Fig. 2 has been accomplished, with a yield of over 150 mg (see Fig. 3 legend for methods). The synthetic peptide had vasorelaxant activity paralleling that of partially purified natriuretic factor (Fig. 3). Its activity was enhanced by allowing it to oxidize slowly, and the activity of the fully oxidized form was very similar to that of the purified peptide (Fig. 3), suggesting the Cys 4–Cys 20 disulphide configuration proposed in Fig. 2.

The synthetic peptide was natriuretic both in the intact rat (Table 1) and in the isolated rat kidney (Table 2). In the latter, perfused in the absence of vasoconstrictors, the peptide increased renal vascular resistance, filtration fraction and glomerular filtration rate (Table 2). In contrast, in the isolated kidney pre-contracted with endogenously generated angiotensin, the peptide decreased vascular resistance (not shown). These effects are similar to those previously described for partially purified peptide, confirming our conclusion that the ANF can have renal vasoconstrictive or vasorelaxant effects

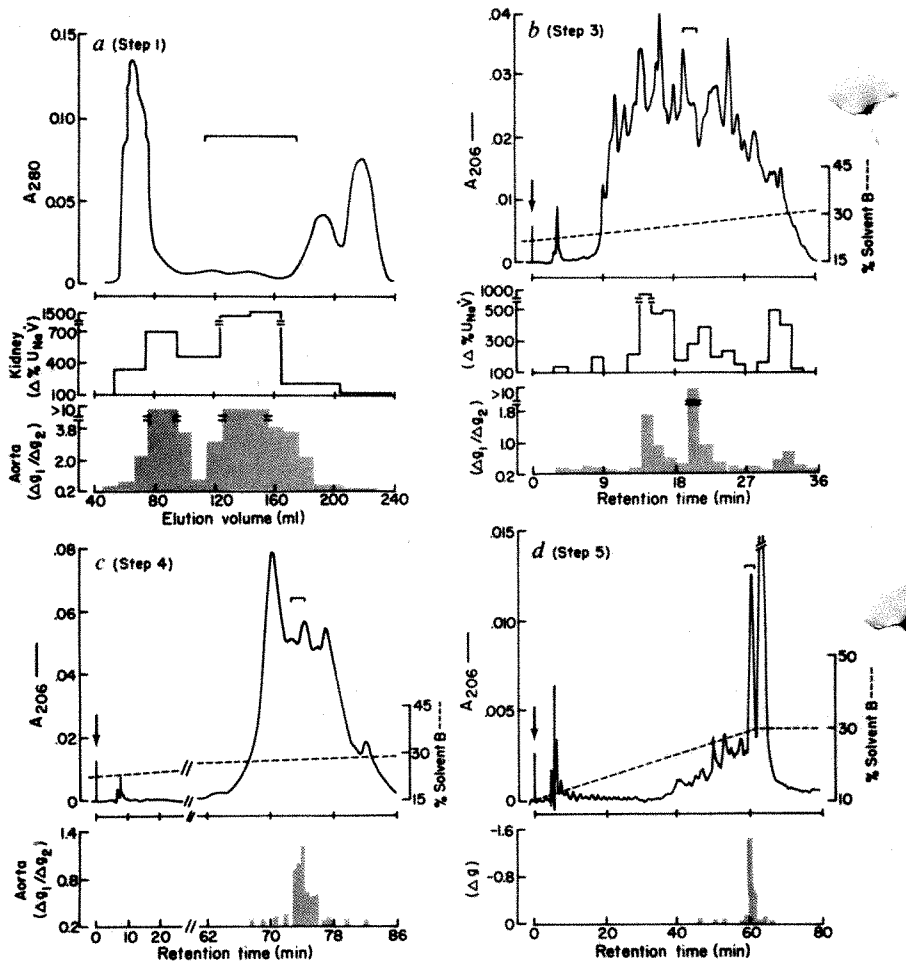


Fig. 1 Purification of natriuretic and vasorelaxant factors from rat atria. Right and left atria from 200 male Wistar rats were homogenized in 8 vols of 1 M acetic acid containing 1 mM phenylmethylsulphonyl fluoride, 3 mM EDTA and 5 μM pepstatin A, centrifuged (10,800*g* for 30 min), and the pellet rehomogenized in 4 vols of buffer. The pooled supernatant was neutralized with ammonium hydroxide[1], centrifuged and lyophilized. *a*, Step 1: gel filtration on a 2.5×45 cm column of Sephadex G-50 (fine) equilibrated with 1 M acetic acid. The extract was reconstituted with 6 ml buffer, centrifuged and applied to the column; flow rate was 0.63 ml min^{-1} and 15.9-min fractions were collected. Aliquots of each fraction were dried (Savant Speed-Vac concentrator), reconstituted in phosphate-buffered saline and assayed for natriuretic activity (0.3 ml) in the intact rat[1] and for vasorelaxant activity (50 μl) in rabbit aortic rings[10]. Rings were first contracted with 2.9×10^{-9}M Ile^5-angiotensin II, then washed, allowed to return to baseline tension and contracted a second time with 1.5×10^{-8}M angiotensin II in the presence of unknown sample; results are expressed as the ratio of the contractile force (Δg tension) generated by the first and second contractions ($\Delta g_1/\Delta g_2$). Six additional extracts were purified similarly and the lyophilized low-molecular-weight region (horizontal bracket) from each was reconstituted with 0.1% aqueous trifluoroacetic acid (TFA), pooled and centrifuged. Step 2 (not shown): one-sixth of the pooled material (representing 1,400 rat hearts), adjusted to 15% acetonitrile (CH_3CN), was applied to a 0.39×30 cm μBondapak C_{18} column, using a Waters U6K injector and solvent delivery system, and eluted at a rate of 0.1 ml min^{-1} with a linear gradient of solvents A (0.1% TFA) to B(CH_3CN) from 85:15 to 45:55 over 40 min. Aliquots of fractions were assayed for natriuresis in the isolated kidney[6,11] and for vasorelaxant activity as described above. A broad region of coincident natriuretic and vasorelaxant activity eluted between 16 and 20 min. The active fractions from six successive runs were pooled and dried overnight. *b*, Step 3; re-chromatography of the step 2 material on the C_{18} column. The sample was reconstituted in solvents A/B 78:22, and chromatographed (in 12 separate applications) at 1.0 ml min^{-1} using a gradient of 22–34% B over 48 min. Aliquots of fractions were tested for natriuretic (100 μl) and vasorelaxant (30 μl) activities as described for step 2. Fractions from each of the three active peaks were pooled and dried overnight. The combined second peak (bracket) from the 12 runs was purified further. *c*, Step 4: re-chromatography of the second peak from step 3. The sample was reconstituted in A/B 77:23, applied to the C_{18} column and eluted at 0.4 ml min^{-1} using a gradient of 23–29% B over 90 min. The bracket indicates fractions with vasorelaxant activity that were pooled from each of six applications. *d*, Step 5: HPLC of the step 4 material on a 0.39×30 cm μBondapak CN column. The solvent system used was A = 0.1% TFA in H_2O and B = 0.055% TFA in CH_3CN. The sample was reconstituted in A/B 90:10, and chromatographed (in three separate applications) at 0.6 ml min^{-1} using a gradient of 10–30% B over 60 min. Vasorelaxant activity was determined by the reduction in tension (Δg) produced in histamine-contracted aortic rings (see Fig. 3 legend for methods). A portion of the active peak (bracket) was re-chromatographed (not shown), giving a single detectable UV peak (retention time 60 min); the remainder was used for sequencing.

depending on the presence of vasoconstrictors[6]. The renal vasoconstrictive effect seems to be exerted preferentially in the efferent arteriole, since it leads to an increase in glomerular filtration rate and in the filtered load of sodium. This latter effect can account for the natriuresis observed in the isolated kidney (Table 2). The vasorelaxant effect predominates in the pre-contracted isolated kidney and possibly in intact animals, leading to an increase in renal blood flow[7,15] and a shift of flow to the inner medulla[15], phenomena known to induce natriuresis. Thus, the available data support the view that the natriuretic effect can be explained by renal haemodynamic actions[6]. Whether this peptide has, in addition, a direct tubular action remains to be demonstrated.

The synthetic peptide, like partially purified preparations, also has potent relaxant effects on the rabbit aorta pre-contracted with histamine (Fig. 3), noradrenaline[8–10], angiotensin II[10] and K^+-depolarization[10] (not shown). No vasoconstrictive effect was detected in aortic rings. The ability to antagonize vasoconstrictors might explain the lowering of blood pressure by atrial extracts[1]. Although no consistent effect on blood pressure was observed in assay rats, preliminary studies in anaesthetized dogs show that constant infusion of the synthetic peptide (at 0.1 μg per kg per min) causes a 10–15% fall in mean arterial pressure.

We have reported here the first synthesis of a natriuretic and vasoactive factor present in mammalian atria. It would be premature to conclude that the structure proposed (Fig. 2) represents the complete sequence of the naturally occurring peptide. The large-molecular-weight active substance in atrial extracts

Table 2 Effects of synthetic auriculin on renal function in isolated perfused rat kidney

	Control	Experimental
GFR (ml min^{-1})	0.43±0.05	0.63±0.03*
FF	0.014±0.002	0.021±0.001*
RVR (mm Hg ml^{-1} min)	2.9±0.1	3.9±0.3*
$\dot{V}$ (μl min^{-1})	19.8±4.8	97.6±19.4*
FL_{Na} (μequiv min^{-1})	60.2±7.9	90.1±5.2*
T_{Na} (μequiv min^{-1})	60.0±7.6	84.2±4.8*
$U_{Na}\dot{V}$ (μequiv min^{-1})	0.66±0.35	6.01±1.99*
FE_{Na} (%)	0.97±0.38	6.6±2.0*
$U_K\dot{V}$ (μequiv min^{-1})	0.44±0.19	1.46±0.16*
FE_K (%)	19.8±5.9	52.1±6.2*

Functioning isolated rat kidneys were perfused in a closed-circuit system with 60 ml of oxygenated perfusate (Krebs–Henseleit bicarbonate buffer, 7.5 g per dl albumin, glucose and amino acids) at an effective perfusion pressure of 90–100 mm Hg (refs 6, 11). After control clearance periods, 150 ng of synthetic peptide were added to the perfusate. Effects on each parameter were noted immediately and reached a maximum during the third 10-min clearance period; these peak values are given as the experimental data. GFR, glomerular filtration rate; FF, filtration fraction; RVR, renal vascular resistance; $\dot{V}$, urine flow rate; FL_{Na}, filtered load of sodium; T_{Na}, tubular reabsorption of sodium; $U_{Na}\dot{V}$, urinary sodium excretion rate; FE_{Na}, fractional sodium excretion; $U_K\dot{V}$, urinary potassium excretion rate; FE_K, fractional potassium excretion. Results are the mean ±s.e. of four kidneys.

*$P<0.05$ compared with control (Student's *t*-test).

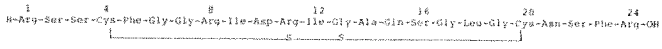


Fig. 2 Amino acid sequence of pure auriculin A. The sequence was determined from 1 nmol of pure protein (step 5, Fig. 1*d*) using the Applied Biosystems 470A gas-phase sequencer. Phenylthiohydrazine amino acids were identified with a Beckman 335T HPLC, using a 0.46×25 cm IBM CN column and a gradient slightly modified from that of Hunkapiller and Hood[17]. The effect of slow oxidation on the biological activity of the synthetic peptide (see text and Fig. 3 legend) suggests the Cys 4–Cyc 20 disulphide configuration shown.

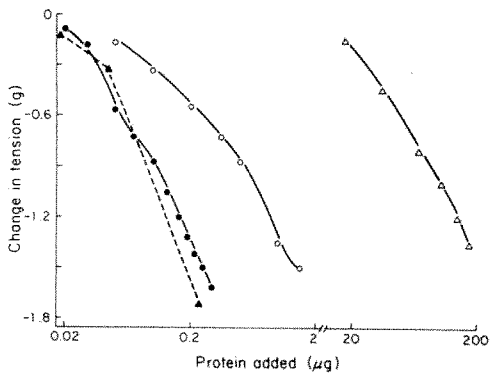


Fig. 3 Comparison of the vasorelaxant activity of purified and synthetic auriculin A on histamine-contracted rabbit thoracic aortic rings. Solid-phase synthesis of the peptide shown in Fig. 2 was performed on a Biosearch SAM II automated peptide synthesizer using t-BOC amino acids. Following repetitive deblocking cycles with TFA, subsequent couplings of amino acids were achieved with a water-soluble carbodiimide. After removal from the polystyrene resin with liquid HF, the synthetic peptide was reduced, purified and slowly re-oxidized[19]. Peptide was dissolved in 2 M acetic acid containing 10 mM β-mercaptoethanol and applied to a 2×96 cm column of Sephadex G-25 (fine) equilibrated with the same buffer (flow rate 0.335 ml min^{-1}, 14.9-min fractions). The major UV (250 nm)-absorbing peak (elution volume 172.5 ml) was lyophilized, resuspended in 0.05 M ammonium acetate, *p*H 7.2 (at 50 μg peptide per ml), allowed to slowly re-oxidize by exposure to air at 4°C in the dark for 60 h and again lyophilized. This material was compared with partially purified (step 1) and purified (step 5) ANF in its ability to relax histamine-contracted aortic rings. Rings were suspended in 10 ml aerated Kreb's buffer under 1.5 g passive tension, pre-contracted with 6×10^{-6} M noradrenaline, washed and allowed to return to baseline tension, as described[10]. A sustained contraction was induced with 5 μM histamine; increasing amounts of purified or synthetic peptide were then added in cumulative fashion. Change in tension is related to the cumulative amount of protein added. Protein concentration was determined by the method of Lowry *et al.*[18] (for step 1) or by dry weight. Purified peptide: Δ, step 1; ▲, step 5. Synthetic peptide: ○, before oxidation; ●, after oxidation.

might represent a precursor, and several low-molecular-weight forms with similar biological activities have been identified[12–14] (see also Fig. 1). We have also detected a form comprising the peptide described here plus an additional C-terminal tyrosine ('auriculin B', unpublished data). Multiple peptides might arise through intracellular processing or as artefacts formed during purification. Our peptides could be fragments of the larger ones purified earlier[12,13]; except for the presence of only a single cysteine in one report[12], the available amino acid composition data are consistent with this possibility. In any event, the present studies demonstrate unequivocally that both the natriuretic and the haemodynamic effects induced by atrial extracts can be accounted for by a single peptide or class of peptides.

An interesting feature of auriculin A is that its C-terminal dipeptide is identical to that of bradykinin. If this C-terminus (rather than Phe-Arg-Tyr) occurs *in vivo*, the peptide might be a substrate for angiotensin-converting enzyme (kininase II), the dipeptidyl hydrolase responsible for the formation of angiotensin II and inactivation of kinins. If so, the secreted peptide might be activated, inactivated or altered in its biological activities on passage through the lung, kidney or other tissues rich in converting enzyme. Although there is so far no proof that this substance is released into the circulation, its reported association with specific membrane-bound atrial granules[4,16] makes it likely. Its potent natriuretic and vascular actions suggest a possible major role in fluid volume and blood pressure haemostasis.

Note added in proof: After submitting this report we learned of two earlier papers describing 28-residue rat[20] and human[21] atrial peptides with three additional N-terminal amino acids and the C-terminal Tyr found in auriculin B. Although the ANF precursor sequence (see following papers) predicts the auriculin B as a likely product of proteolytic processing, further work is required to clarify this point.

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Cloning and sequence analysis of the cDNA for the rat atrial natriuretic factor precursor

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Atrial extracts contain factors which induce potent natriuresis[1,2] changes in renal haemodynamics[3], and relax pre-contracted vascular smooth muscle[3–5]. Low-molecular-weight peptides which mimic these actions have now been purified by several groups[1,2,6–12], including ours (see accompanying paper[9]), and higher-molecular-weight proteins with similar but less potent biological activities have also been identified and are presumed to be precursors[6,7,9–12]. If released into the circulation, these peptides, collectively called atrial natriuretic factor (ANF), may play a significant part in blood-pressure homeostasis, regulation of extracellular fluid volume and as antagonists to the hypertensive effects of the renin–angiotensin system and other hormonal and neurotransmitter systems. We describe here the isolation and characterization of rat atrial cDNA clones which encode ANF. Nucleotide sequence analysis shows that auriculin[9] corresponds to the 25 amino acids located close to the C-terminus of a 152-amino acid ANF precursor. Analysis of the *in vitro* translation products of precursor ANF mRNA suggests that multiple forms of the precursor may exist.

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                                  4   5   6   7   8   9  10  11  12  13
Amino acids                      Cys Phe Gly Gly Arg Ile Asp Arg Ile Gly

Possible codons    5' TGY TTY GGN GGN CGN ATY GAY CGN ATY GGN 3'
                                              AGR ATA     AGR ATA

                   probe a     3' ACR AAR CCN CCN GC  5'
REGION 1
                   probe b     3' ACR AAR CCN CCN TC  5'

                   probe c                          3' TA(R/T) CTR GCN TA(R/T) CC  5'
REGION 2
                   probe d                          3' TA(R/T) CTR TCN TA(R/T) CC  5'
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Fig. 1 Synthesis and use of oligonucleotide probes. The sequence of amino acids 4–12 of rat auriculin[9] is shown along with the sequence of the four oligonucleotide mixtures, a and b for region 1, and c and d for region 2. R = A or G, Y = T or C, N = A, G, T or C. Each oligonucleotide mixture was synthesized on a Biosearch SAM I oligonucleotide synthesizer by a modification of the standard phosphotriester method using mesitylenesulphonyl chloride in the presence of *N*-methylimidazole as condensing reagents[28,29] and purified by polyacrylamide gel electrophoresis. Double-stranded cDNA was synthesized from rat atrial poly(A)$^+$ RNA. After the sequential addition of *Eco*RI and *Sal*I oligonucleotide linkers[13] the cDNA was ligated to the plasmid expression vector pUC9 (ref. 14) and transformed onto *Escherichia coli* MC1061 (ref. 30). 5 μg of rat atrial poly(A)$^+$ RNA yielded about 25 ng of cDNA, size-selected to greater than 300 base pairs (bp), and gave a library of about 200,000 independent recombinants. These were plated on Millipore Triton-free nitrocellulose filters, replica-plated and the library was stored frozen on glycerol-impregnated filters at −70 °C (refs 18, 19). Replica filters of the library were processed for hybridization[15–17]. After baking, the filters were washed overnight at 68 °C with shaking in a large volume of 3 × SSC (20 × SSC = 3 M NaCl, 0.3 M sodium citrate *p*H 7.5), 0.1% SDS and then prehybridized in 6 × SSC, 0.1% SDS, 1 mM EDTA, 5 × Denhardt's solution[31], 0.05% sodium pyrophosphate at 50 °C for at least 2 h (ref. 16). The filters were then hybridized with 2.5×10^6 c.p.m. (1 pmol ml^{-1}) ^{32}P-labelled oligonucleotide mixture per filter, in hybridization solution containing 100 μg ml^{-1} tRNA at 45 °C for 1 h in a shaking water bath. The thermostat was then lowered to 25 °C and the bath allowed to equilibrate overnight. The filters were washed twice in 6 × SSC, 0.1% SDS at room temperature for 15 min, then washed at 35 °C (for probes c and d) or 39 °C (for probes a and b) for 1–2 min. The final washing temperature was obtained from the empirical formula of Suggs *et al.*[32], $T_d = 4(G+C) + 2(A+T)$. The filters were then dried and autoradiographed with intensifying screens.

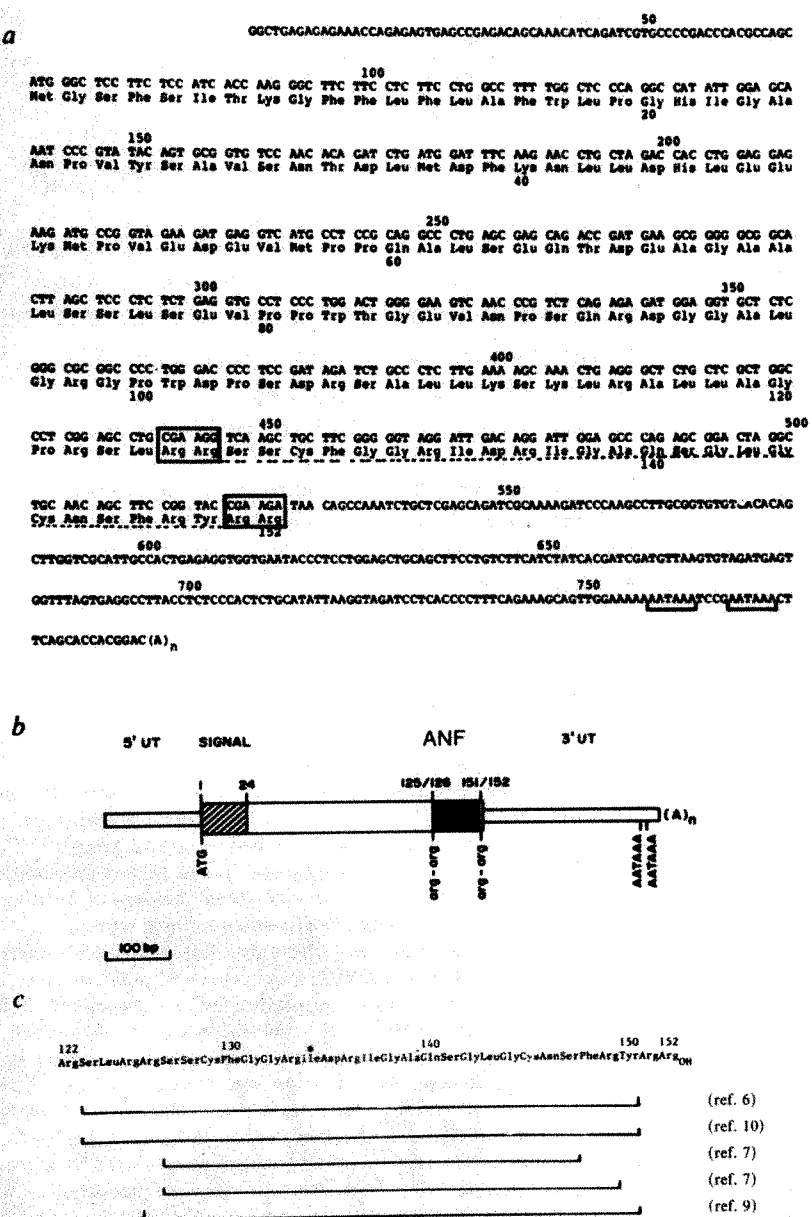


Fig. 2 *a*, Nucleotide sequence of precursor ANF. The nucleotide sequence was established by the chain-termination method[33] using cDNA fragments subcloned into the M13 vectors mp8 and mp9 (ref. 34). All sequences were determined on both strands with the exceptions of nucleotides 755–790. The sequence for this region was obtained from another cDNA clone isolated using pNF1 as a hybridization probe. The basic Arg-Arg dipeptides, which are implicated in the processing of precursor ANF, are boxed, while the two AATAAA sequences involved in polyadenylation are underlined. *b*, Schematic representation of the structure of precursor ANF. The precursor sequence, amino acids 1–152, is shown with the putative signal peptide marked by cross-hatching and the auriculin sequence marked by stippling. The positions of the two Arg-Arg dipeptides (amino acids 125–126 and 151–152), which are potential processing sites, are shown. The 5′- and 3′-untranslated regions (UT) are marked and the two putative polyadenylation signals, AATAAA, are identified. *c*, Amino acid sequences of rat and human atrial peptides. The sequences of other biologically active peptides[6,7,9,10] are shown. The Ile at position 134 (marked with an asterisk) is a Met in the human sequence[10].

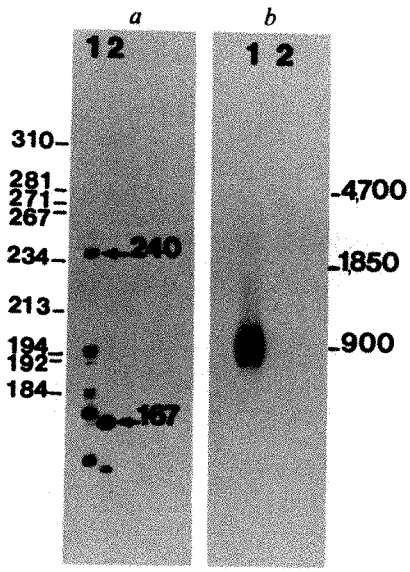


Fig. 3 Size determination of precursor ANF mRNA. *a*, Primer extension. Five μg pNF1 DNA were digested with *Bgl*II, treated with bacterial alkaline phosphatase and 5'-end-labelled with [γ-^{32}P]ATP by polynucleotide kinase. The kinase was heat-inactivated, the DNA digested with *Dde*I and the 167-bp *Dde*I–*Bgl*II fragment (nucleotides 3–170), ^{32}P-labelled at the *Bgl*II end only, was recovered from a polyacrylamide gel. One-fifth of the eluted DNA fragment was co-precipitated with 1 μg rat atrial poly(A)$^+$ RNA[35,36], resuspended in 40 μl 80% formamide, 0.4 M NaCl, 40 mM PIPES *p*H 6.4, 1 mM EDTA (refs 37, 38), heated to 68 °C for 10 min and hybridized at 55 °C for 3 h. Following ethanol precipitation the annealed primer–template complex was incubated with unlabelled deoxynucleoside triphosphates and reverse transcriptase and the size of the primer extension products estimated by electrophoresis on a 5% acrylamide, 7 M urea gel. Lane 1, primer-extension products; lane 2, unextended primer fragment. Sizes of markers fragments are given in base pairs. The full-length primer extension product (240 nucleotides) is 73 nucleotides longer than the primer fragment. Since the *Dde*I priming site is 3 nucleotides from the end of pNF1, the cDNA clone is about 70 nucleotides short of being full length. A strong intermediate band of about 195 nucleotides is also seen and may represent a region of secondary structure in the mRNA. *b*, Northern blot analysis. Poly(A)$^+$ RNA (2 μg) isolated from rat atrium, or rat ventricle, was fractionated by electrophoresis on a 1.4% agarose gel containing methyl mercuric hydroxide[39]. The RNA was transferred to a nitrocellulose filter[40] and probed with nick-translated pNF1 DNA. Lane 1, atrial poly(A)$^+$ RNA; lane 2, ventricular poly(A)$^+$ RNA. The positions of 28S and 18S rRNA markers are given in bases.

The sequences of atrial natriuretic peptides reported to date differ slightly in length at both the N- and C-termini, which may be a reflection of variable proteolytic processing of a precursor. We used these amino acid sequences to design oligonucleotide probes to screen a rat atrial cDNA library cloned in the vector pUC9 (refs 13, 14) (Fig. 1). These probes were synthesized to correspond to two non-overlapping regions of the peptide (Fig. 1). Due to the degeneracy of the codons, we chose to synthesize two oligonucleotide pools for each region. Thus, region 1 was covered by two tetradecamer oligonucleotide pools, a and b, each consisting of 64 sequences, while region 2 was covered by another two tetradecamer pools, c and d, each consisting of 72 sequences.

The pools of oligonucleotides were used to screen the atrial cDNA library by colony hybridization[15–17]. Four replica filters were prepared from each master filter[18,19], and each replica was probed with one of the oligonucleotide pools. A colony was considered positive if it hybridized with one of the region 1 and one of the region 2 probes. One clone was picked as the most likely candidate for encoding auriculin as it not only hybridized very strongly to the oligonucleotide probes, but also represented an atrium-specific sequence. This was demonstrated by its hybridization to a random primed atrial, but not ventricular, cDNA probe.

Nucleotide sequence analysis confirmed that this clone (pNF1) encodes an auriculin precursor. When the atrial cDNA library was re-screened with this cDNA insert, approximately 0.5% of the colonies hybridized, showing that ANF is a major species in rat atrial mRNA. The complete nucleotide sequence and structure of the precursor is shown in Fig. 2. A single open reading frame encoding a 152 amino acid sequence extends from an ATG initiation codon at nucleotide 69 to a TAA termination codon at nucleotide 525. Within this reading frame the sequence of the various forms of biologically active atrial natriuretic peptides can be identified (Fig. 2*c*). The sequence in Fig. 2*a* has a 5'-untranslated region of 68 nucleotides and a 3'-untranslated region of 266 nucleotides. Two potential polyadenylation signals, AATAAA (ref. 20), are seen in the 3'-untranslated region. Multiple polyadenylation sites have also been observed in other genes[21,22].

To confirm that the cDNA clone, pNF1, represents an essentially full-length transcript of precursor ANF mRNA, we isolated a restriction enzyme fragment from pNF1 and used it to prime cDNA synthesis from atrial poly(A)$^+$ RNA. The results (Fig. 3*a*) show that pNF1 is about 70 nucleotides short of a full-length cDNA clone. The mRNA for precursor ANF is therefore about 850 nucleotides long (excluding the poly (A) tail). This conclusion is supported by the results of a Northern blot (Fig. 3*b*), in which pNF1 hybridizes to an atrial mRNA of approximately 900 nucleotides but is not detected with ventricular mRNA (Fig. 3*b*).

Other evidence supports our identification of the reading frame of the ANF precursor. The precursor has been proposed to be a secretory protein, found in atrial granules, and would therefore be expected to have a signal peptide, and indeed, the sequence following the proposed initiation codon (Fig. 2*a*) encodes a stretch of amino acids, starting at position 9 which, though unusually rich in phenylalanine, has the hydrophobic character of a signal peptide[23]. Cleavage of this sequence could occur after either the glycine at position 23 or the alanine at position 24 (the preferred amino acids at the signal peptide cleavage site), producing a signal peptide of normal length. Eukaryotic genes do not have a ribosome-binding site sequence like that seen in prokaryotes, although a consensus sequence, CC$\frac{A}{G}$CCATGG, is found around the initiation codon[24]. In the case of precursor ANF, this sequence is CCAGCATGG. Finally, the identification of the reading frame is supported by the fact that the cDNA clone is similar in size to the mRNA, and that the molecular weight of the precursor protein as predicted from the sequence matches closely with the observed size (see below). A schematic version of precursor ANF is shown in Fig. 2*b*.

The diversity of previously identified amino acid sequences encoding natriuretic peptides (see Fig. 2*c*) does not allow precise determination of the sites at which precursor ANF is processed. However, the sequence of auriculin is flanked on both sides by basic dipeptides (Arg-Arg at positions 125–126 and 151–152) which have been identified in other hormone precursors as proteolytic processing sites[25]. The varying lengths of the active peptides identified by the different groups could result either from the precursor being processed at sites other than Arg–Arg or from an artefactual proteolytic cleavage of the precursor during purification. The Arg-Arg dipeptide at the C terminus of auriculin is immediately followed by the TAA termination codon and, as such, is similar to that of the corticotropin-releasing factor precursor[26].

The predicted molecular weight (MW) of precursor ANF is 16,720. To determine the actual precursor size, atrial mRNA encoding precursor ANF was purified by hybrid selection[27], using pNF1 DNA, and translated *in vitro*. The selected translation products were then analysed by two-dimensional gel electrophoresis and compared with the total translation products from atrial and ventricular mRNA (Fig. 4). Translation of the

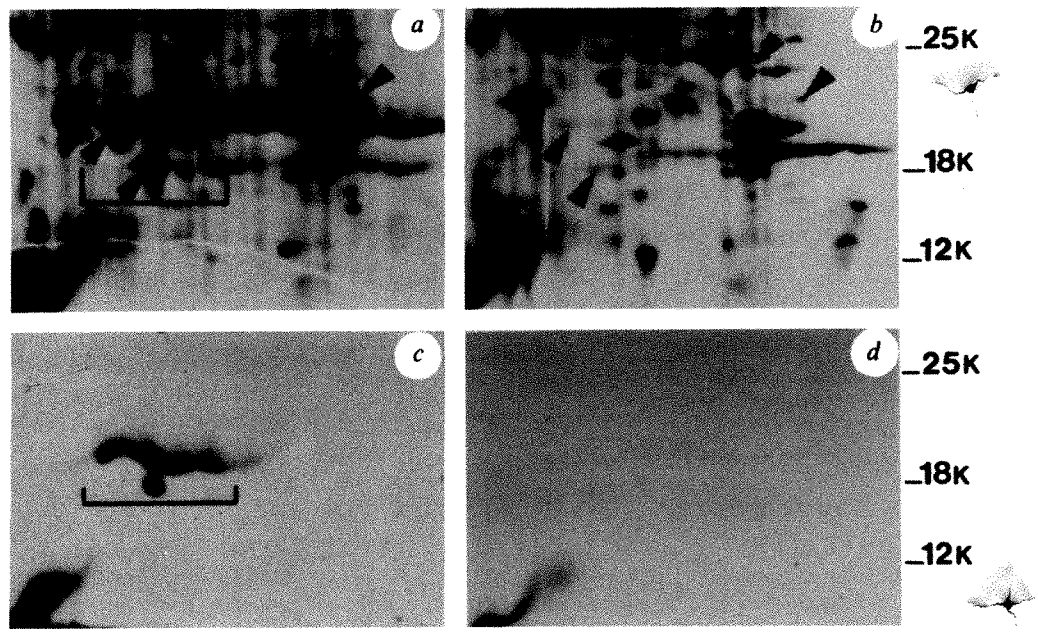


Fig. 4 Two-dimensional gel fractionation of cell-free translation products encoded by poly(A)$^+$ RNA isolated from: *a*, rat atria; *b*, ventricle, or selected by hybridization[27] of atrial (*c*) or ventricular (*d*) poly(A)$^+$ RNA to pNF1 DNA. 5 μg of plasmid pNF1 DNA were immobilized on 1-cm^2 nitrocellulose disks and hybridized with 5 μg of poly(A)$^+$ RNA for 3 h at 50 °C in 20 mM PIPES, *p*H 6.4, 1 mM EDTA, 65% formamide, 5 × SSC, 0.1% SDS. Filters were then washed extensively in 10 mM Tris-Cl *p*H 7.5, 0.15 M NaCl, 1 mM EDTA, 0.1% SDS at 70 °C and at 37 °C in the same buffer without SDS. Hybridized RNA was eluted in H_2O at 100 °C in the presence of 50 μg tRNA for 1 min, quick-frozen at −70 °C, thawed and precipitated by the addition of 2 vols absolute ethanol. Hybrid-selected RNA and total poly(A)$^+$ RNA was translated using a commercially available rabbit reticulocyte lysate system in the presence of 250 μCi ml^{-1} ^{35}S-methionine. Acid-precipitable radioactivity (1×10^6 c.p.m.) translated from total atrial and ventricular poly(A)$^+$ RNA or 1×10^5 and 5×10^4 c.p.m. of acid-precipitable radioactivity translated from hybrid-selected atrial and ventricular RNA, respectively, was fractionated by two-dimensional gel electrophoresis. The first dimension consisted of isoelectric focusing using a *p*H 3.5–10 gradient[41] while the second dimension was a 15% acrylamide–SDS gel[42]. The gels were equilibrated in 1 M sodium salicylate, dried and fluorographed at −70 °C for 24 h. The electrophoretic positions of several atrium-specific translation products in the 12,000–30,000 MW range are marked by arrows. The translation products encoded by pNF1-hybrid-selected atrial RNA are indicated by brackets.

hybrid-selected RNA yielded at least three proteins with molecular weights of 16,500–18,500 (Fig. 4*c*). These translation products selected by pNF1 represent abundant atrial species which are not present in the ventricle.

The function of the N-terminal portion of the precursor is not clear. Higher-molecular-weight forms of biologically active material have been isolated[4,7,9–11] but they have less potent natriuretic and diuretic effects than auriculin. These activities could result from the proteolytic cleavage of the precursor in the assay system releasing auriculin. The N-terminal fragment lacking auriculin has not been identified *in vivo*, and therefore has no biological activity ascribed to it. Knowledge of the sequence of the entire precursor will allow us to express the complete ANF precursor, or defined fragments of it, in yeast or bacteria, and to investigate their biological activities.

Note added in proof: We have sequenced the primer extension product and confirmed the sequence from nucleotides 7 to 54. Nucleotides 1–6 are, however, incorrect, and probably result from a cloning artefact.

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Structure of rat atrial natriuretic factor precursor deduced from cDNA sequence

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The atrium of the heart contains peptides, termed atrial natriuretic factors (ANFs), diuretic[1] and smooth-muscle-relaxing[2–4] activities. In view of its potent effects on salt metabolism in the kidney and on vascular smooth muscle, ANF is considered to play an important part in the control of fluid volume and vascular function. Several different ANF peptides varying in size have been isolated[3–10] and their amino acid sequences determined[4,6–10]. Analysis of the

sequences of the peptides suggests that they are derived by proteolysis from the same precursor. To examine this hypothesis, we have cloned cDNAs of the ANF precursor using rat atrial mRNA, determined its nucleotide sequence and deduced its amino acid sequence. The ANF precursor consists of 152 amino acid residues including a putative signal peptide sequence. This sequence contains the amino acid sequences of all the ANF peptides reported to date[4,6–10].

For use as hybridization probes, two pentapeptide segments of rat ANF peptide[4] which gave the least numbers of possible combinations of codons were chosen for constructing 14-mer mixed oligonucleotides designated oligo I and oligo II (Fig. 1*a*). The ability of the synthetic oligonucleotides to specifically hybridize with mRNA for ANF was tested, using oligo I as a primer, by reverse transcription of poly(A) RNA isolated from rat atrium (Fig. 1*b*). Poly(A) RNA from ventricle was used as a control. Following urea-polyacrylamide gel electrophoresis, only one major cDNA band corresponding to 460 nucleotides in length was produced from atrial mRNA, but not from ventricular mRNA, suggesting specific hybridization of oligo I to ANF mRNA.

A library of rat atrial cDNA clones was constructed using the cloning vector of Okayama and Berg[11] (see Fig. 2 legend for experimental details). By screening with oligo I, 56 hybridization-positive clones were selected from 900 transformants. Using oligo II as the second probe, candidates were narrowed down to eight clones. All eight clones had cDNA inserts of indistinguishable sizes with common restriction endonuclease sites for *Pst*I, *Xho*I, *Hin*cII and *Bgl*II.

The nucleotide sequence analysis of one of these clones, pOB8, by the method of Sanger *et al.*[12–14] revealed that it lacks a 5′-noncoding sequence as well as the ATG initiation triplet.

A clone with a longer cDNA insert, pGH33, was isolated from a second atrial cDNA library prepared by the method of Gubler and Hoffman[15] (see Fig. 2 legend). Its nucleotide sequence, determined by the methods of Sanger *et al.*[12–14] and of Maxam and Gilbert[16], was in agreement with that of pOB8 in the overlapping region. The complete nucleotide sequence of the ANF cDNA clones consisting of 786 residues is presented in Fig. 3.

The nucleotide sequence from residue 376 to residue 450 corresponds exactly to the previously determined amino acid sequence of ANF peptide (the boxed region in Fig. 3); it allowed us to define the reading frame for the entire ANF precursor and, thus, deduce its amino acid sequence. The distance from the 5′ end to the position of oligo I was 461 nucleotides long, which agrees with the observed size (460 nucleotides) of the major cDNA product of the primer-extended reverse transcript of atrial mRNA (Fig. 1*b*). Therefore, the first ATG triplet (nucleotide residues 1–3) proximal to the 5′ end of the sense strand was designated as the initiation codon. An unusually

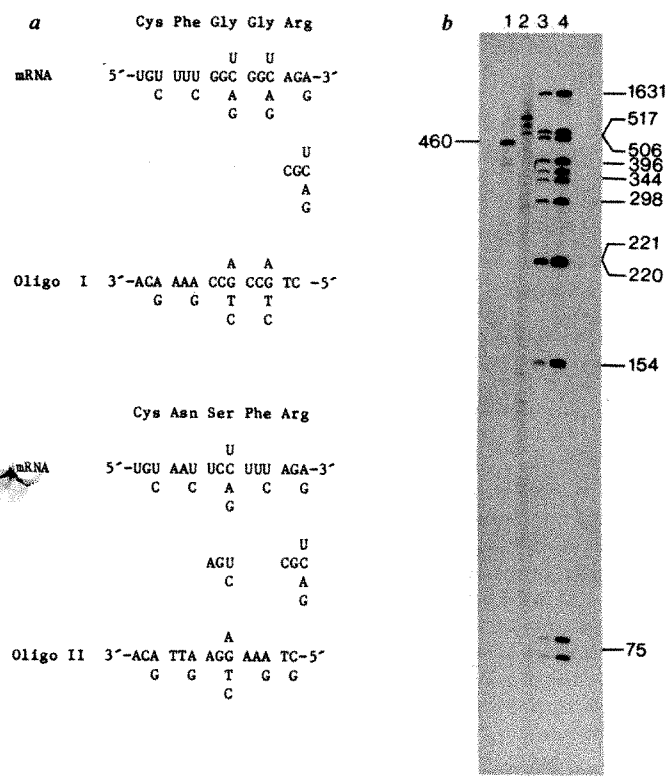


Fig. 1 *a*, Synthetic oligonucleotides used for screening cDNA clones for rat ANF. Mixed 14-mer oligodeoxynucleotides, oligo I and oligo II (custom-synthesized by American Bionuclear) are complementary to all possible coding sequences corresponding to the two pentapeptide sequences which had been found in ANF peptides[4]. Codons for Arg in oligo I and for Ser in oligo II were selected on the basis of the frequency of codon usage in rat mRNA. *b*, Electrophoretic analysis of cDNA products synthesized from poly(A) RNA using oligo I as a primer. Poly(A) RNA was isolated from atria and ventricles of Sprague–Dawley rats by the guanidine isothiocyanate–CsCl procedure[22] followed by oligo(dT)-cellulose chromatography. cDNA was synthesized from 20 μg of poly(A) RNA obtained from atria (lane 1) or ventricles (lane 2), using ^{32}P-labelled oligo I (0.125 μg, 1.5×10^8 c.p.m. μg^{-1}) as a primer for reverse transcriptase reaction, and was electrophoresed on an 8% polyacrylamide gel containing 7 M urea[23]. *Hin*fI digests of pBR322 (NEN) were labelled with ^{32}P and used as markers for the sizes and radioactivity of synthesized cDNAs (lane 3, 1,200 c.p.m.; lane 4, 6,000 c.p.m.).

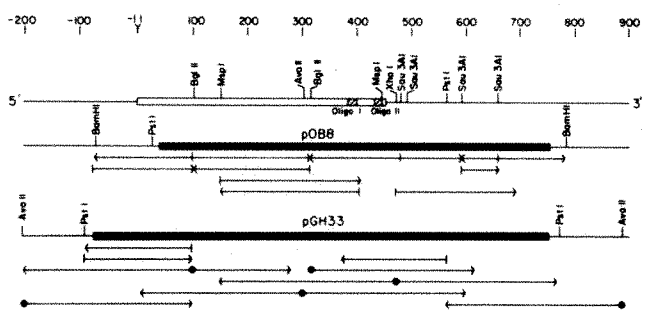


Fig. 2 Sequencing strategy of ANF cDNA. Clones pOB8 and pGH33 were isolated from two cDNA libraries prepared independently by the procedures of Okayama and Berg[11,24] (method A) and Gubler and Hoffman[15] (method B) respectively.

Methods: A: cDNA–plasmid recombinant molecules were prepared using 4 μg of poly(A) RNA from rat atria, 2.2 μg of dT-tailed pcD vector[11] and dG-tailed linker. *Escherichia coli* N-38, a substrain of HB101, was used for transformation and ampicillin-resistant transformants were screened by colony hybridization[25] at 37 °C or 41 °C with oligo I (Fig. 1*a*) labelled with ^{32}P at the 5′ end. The hybridization-positive clones were re-screened with oligo II (Fig. 1*a*) at a hybridization temperature of 31 °C or 37 °C. B: The first cDNA strand was synthesized using 10 μg of poly(A) RNA as template which had been denatured by 10 mM of methylmercuric hydroxide[26]. The RNA was replaced by the second cDNA strand using a mixture of RNase H, DNA polymerase I and DNA ligase. The double-stranded cDNA thus obtained was tailed with dCTP, annealed to *Pst*I-cleaved and dG-tailed pBR322 and introduced into *E. coli* N-38. Tetracycline-resistant transformants were screened by colony hybridization with ^{32}P-labelled *Msp*I fragment of pOB8 cDNA as a probe. The nucleotide sequences of the plasmid inserts pOB8 and pGH33 (solid boxes) were determined both by the dideoxy terminator method of Sanger *et al.*[12] (sequencing direction indicated by arrows with a short vertical line) and by the method of Maxam and Gilbert[16] (indicated by arrows with solid circles). In the former, restriction fragments were subcloned into M13mp8 or M13mp9 (ref. 13) and Klenow fragment of DNA polymerase I or reverse transcriptase (ref. 14) was used in the chain-termination reaction. The open box indicates the coding region flanked by an initiation codon and a termination codon. The hatched regions represent the segments corresponding to synthetic oligonucleotides (oligo I and oligo II) used as probes.

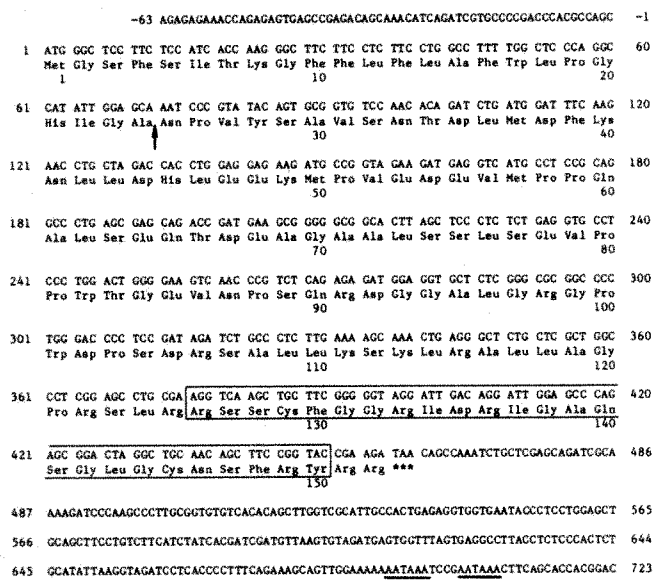


Fig. 3 Nucleotide sequence of rat ANF cDNA. Nucleotides are numbered in the 5′ and 3′ direction beginning with the first residue of the ATG triplet encoding the translational initiation methionine, and the nucleotides on the 5′ side of residue 1 are indicated by negative numbers. The deduced amino acid sequence is shown below the nucleotide sequence. A potential cleavage site for the signal peptide is indicated by an arrow between residues 24 and 25. Potential poly(A) addition signals (AATAAA)[27] are underlined. The boxed region shows the amino acid sequence previously determined by automated Edman degradation of the 25-amino acid ANF peptide[4].

long inverted repeat sequence (nucleotides 467–482) lies immediately downstream from the TAA stop codon.

The nucleotide sequence of rat ANF cDNA reveals that the ultimate precursor of ANF peptide comprises 152 amino acids with a calculated molecular weight of 16,556. The amino-terminal 24-amino acid sequence appears to be a signal peptide[17] as it contains a region which is rich in hydrophobic amino acids with bulky side chains (positions 10–18) and is followed by a region containing amino acids having small side chains[18]. While this work was in progress, Thibault *et al.*[19] reported the amino-terminal sequence of a rat high-molecular-weight ANF, which corresponds with the stretch from Glu 78 to Asp 135 in the precursor sequence deduced in the present study.

The finding of the carboxy-terminal sequence -Arg-Arg (151–152) is intriguing as no active ANF peptide isolated and sequenced to date contains this structure[4,6–9]. The possibility cannot be excluded that the cleavage of this pair of arginine residues may be related to the activation of ANF.

The recent findings linking atrial deficiency of natriuretic activity with hereditary congestive heart failure in a strain of Syrian hamster[20] and with spontaneous hypertension in rat[21] strongly indicate that ANF has an important role in cardiovascular regulation. cDNA for ANF obtained in the present study may be useful in elucidating regulation of ANF mRNA synthesis and its involvement in pathogenesis of such cardiovascular diseases. The deduced amino acid sequence of the precursor of ANF should also facilitate elucidation of the mechanism of synthesis, processing and secretion of ANF.

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Cloning and sequence analysis of cDNA encoding a precursor for human atrial natriuretic polypeptide

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Recent identification of natriuretic–diuretic activity in peptides isolated from human[1] and rat [2–5] atrial tissue implicates them in the control of extracellular fluid volume and electrolytic homeostasis. The presence of multiple forms of the peptides ranging from 3,000 to 13,000 molecular weight (MW) suggests they may all derive from the same precursor[1–6]. The established amino acid sequence of α-human atrial natriuretic polypeptide (α-hANP)[1], a 28-residue peptide with potent natriuretic activity, provided the means to elucidate the structure of the precursor for α-hANP and the gene encoding it. Here we report the cloning and sequence analysis of the cDNA of human atrial mRNA encoding a precursor of α-hANP. The cDNA encodes γ-human atrial natriuretic polypeptide (γ-hANP) of 13,000 MW, whose C-terminal 28 amino acid residues may be processed as α-hANP.

A library of cDNA clones from human atrial poly(A)$^+$ RNA was constructed using the method of Okayama and Berg[7]. To probe cloned cDNA for the precursor of α-hANP, tetradecamer oligodeoxyribonucleotides representing all possible complementary sequences corresponding to amino acids 12–16, Met-Asp-Arg-Ile-Gly, of α-hANP (excluding the third nucleotide residue of the glycine codon) were synthesized by modified triester methods[8] as two pools (oligo I and oligo II). The cDNA library was screened by hybridization with ^{32}P-labelled probes. Twenty-three clones, which hybridized with oligo II but not with oligo I, were isolated from about 40,000 transformants. Plasmids isolated from eight of these clones were submitted to nucleotide sequence analysis and all were found to carry a DNA segment encoding the N-terminal sequence (1–11) of α-hANP. Two plasmids of this group, phANP1, harbouring the longest

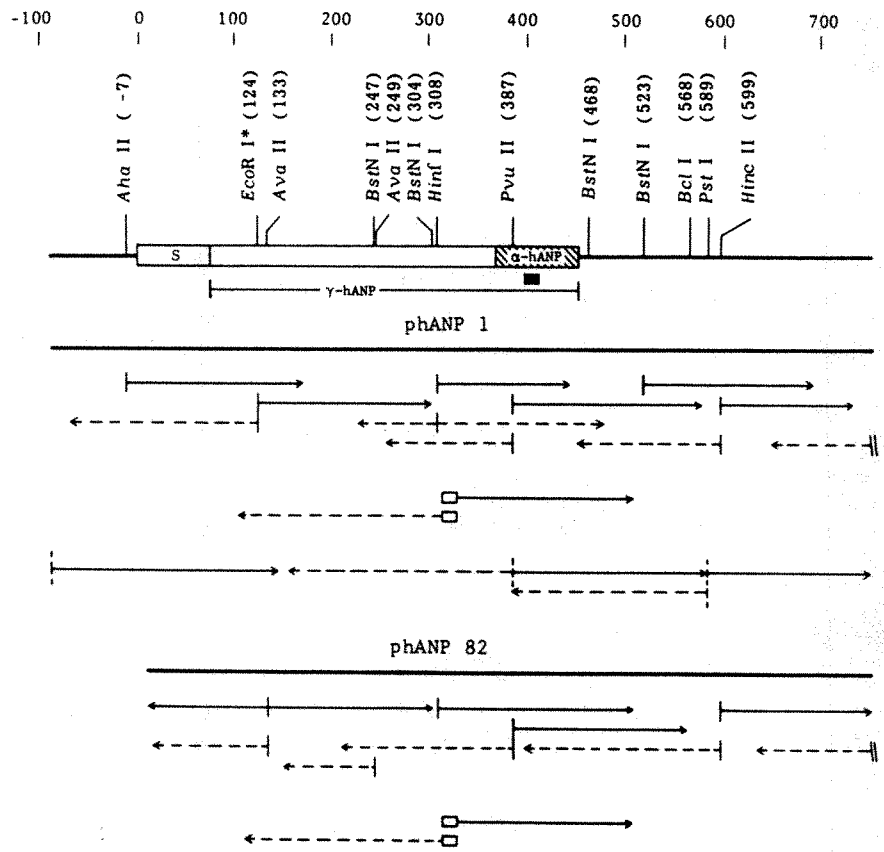


Fig. 1 Strategy of sequencing the cDNA inserts in clones phANP1 and phANP82. The restriction map displays only those restriction endonuclease sites relevant to the present work. They are identified by numbers indicating 5′-terminal nucleotides generated by cleavage. Nucleotides are numbered as in Fig. 2. S, Coding region for putative signal peptide; ▨, coding region for α-hANP; ■, region of the sequence of synthetic oligodeoxyribonucleotides used in probing cloned cDNAs. The direction and extent of sequence determinations are shown by arrows (→, upper strand; ---›, lower strand). The sites of 5′- and 3′-end-labelling are indicated by short vertical lines at the ends of arrows. Double slashes indicate that 5′-end-labelling was done on the sites located on the vector DNA. Dotted lines mean that the transcription primed by the general primers was started from sites located on the pUC8 plasmid[18]. Open boxes at the end of arrows indicate the region of the primer sequence detailed below.

Methods: The clones were obtained as follows. One mg of total RNA was extracted[19] from atria of an 87-year-old female and a 61-year-old male post mortem. A total of 75 μg of poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose binding procedure[20] using 10 mM Tris–HCl buffer, *p*H 7.2 containing 0.5 M $LiCl_2$, 10 mM EDTA and 0.5% SDS as a binding buffer. A cDNA library was constructed by the method of Okayama and Berg[7] using 15 μg of poly(A)$^+$ RNA and 4.2 μg of the vector-primer. *Escherichia coli* WA802 was used for transformation. Approximately 40,000 ampicillin-resistant transformants were obtained per μg of starting mRNA and screened by hybridization[21] at 38 °C for 16 h with the 5′-^{32}P-labelled mixtures of oligo I and oligo II. A second hybridization was done on the 85 positive clones with similarly labelled oligo I and oligo II at 40 °C and 38 °C for 16 h respectively. Plasmids were isolated from 8 clones out of 23 which hybridized with oligo II but not with oligo I. The chain-terminator nucleotide-sequencing method of Sanger *et al.*[22] using oligo II as a primer showed that all 8 plasmids carried an insert which contained the coding sequence corresponding to the first 11 amino acids of α-hANP. Two plasmids, phANP1 and phANP82, having the longest inserts (about 950 bp and 850 bp respectively), were used for further sequencing by the following methods. *a*, Maxam–Gilbert method[23]; *b*, method of Sanger *et al.*[22], using as primers oligodeoxyribonucleotides of the sequences 5′-GATCGATCTGCCCTC-3′ and 3′-CTAGCTAGACGGGAG-5′ for lower and upper strands respectively; *c*, method of Sanger *et al.*[22] after cloning three fragments of the insert generated by digestion with *Pst*I and *Pvu*II endonucleases into pUC8 plasmid[18].

cDNA insert of about 950 base pairs (bp) and phANP82 (about

```
        Met Asp Arg Ile Gly
3′-TAC CT(G/A) GC(T/G/C/A) TA(T/G/A) CC-5′    (oligo I)

3′-TAC CT(G/A) TC(T/C) TA(T/G/A) CC-5′        (oligo II)
```

850 bp), were sequenced according to the strategy indicated in Fig. 1 legend.

Figure 2 depicts the complete nucleotide sequence of the cDNA insert of the phANP1 plasmid which is 843 bp long (excluding poly(A) sequence). All of the 3′ portion of the mRNA upstream of the poly(A) tail[9] was represented in the insert because about 80 consecutive A residues were found at the 3′ end. Most of the 5′ end portion of the mRNA also seems to be represented in the insert, because none of 58 independently isolated transformants had plasmids with an insert appreciably longer than that of phANP1 on cleared lysate analysis[10].

The nucleotide sequence of phANP1 has a single large open reading frame, the first ATG codon appearing as 91–93 nucleotides from the 5′ end (nucleotide residues 1–3 in Fig. 2). A TGA termination triplet is found 151 codons later. The 3′-untranslated region of 300 nucleotides contains the hexanucleotide sequence AATAAA (underlined in Fig. 2), which is known to precede polyadenylation sites in many eukaryotic mRNAs[11].

Figure 2 shows the amino acid sequence deduced from the 151 codons. The sequence of nucleotide residues 370–453, encoding amino acids 124–151, corresponds precisely to the amino acid sequence of α-hANP. In addition, residues 26–151 deduced from the base sequence 76–453 in the cDNA corresponds exactly to the natriuretic polypeptide of MW 13,000, designated γ-hANP, which has been recently identified in human atrial tissue. The complete sequence of 126 amino acids of γ-hANP has been established in our laboratory as starting from H-Asn-Pro-Met-Tyr-Asn- at its N-terminus and ending in -Asn-Ser-Phe-Arg-Tyr-OH at its C-terminus; α-hANP is the C-terminal 28 residues of γ-hANP (details to be published elsewhere).

In the cDNA sequence, the TAC codon for the C-terminal Tyr of γ-hANP is followed by the translational termination codon TGA. Therefore, the protein translated from the reading frame is apparently a precursor for both γ-hANP and α-hANP, and we have called the precursor pre-human atrial natriuretic polypeptide (pre-hANP).

The 25 amino acid residues preceding the γ-hANP sequence exhibit features characteristic of a signal peptide which is usually present at the N-terminal end of precursors of secreted proteins[12,13]. The hydrophobic core[14] corresponding to amino acids 9–20 includes 10 hydrophobic residues and is followed, 5 amino acids later, by an alanine residue at position 25, which very often occurs before the cleaving site of signal peptides[14]. The appreciable sequence similarity between the signal peptides of the precursor for α-hANP and human preproen-

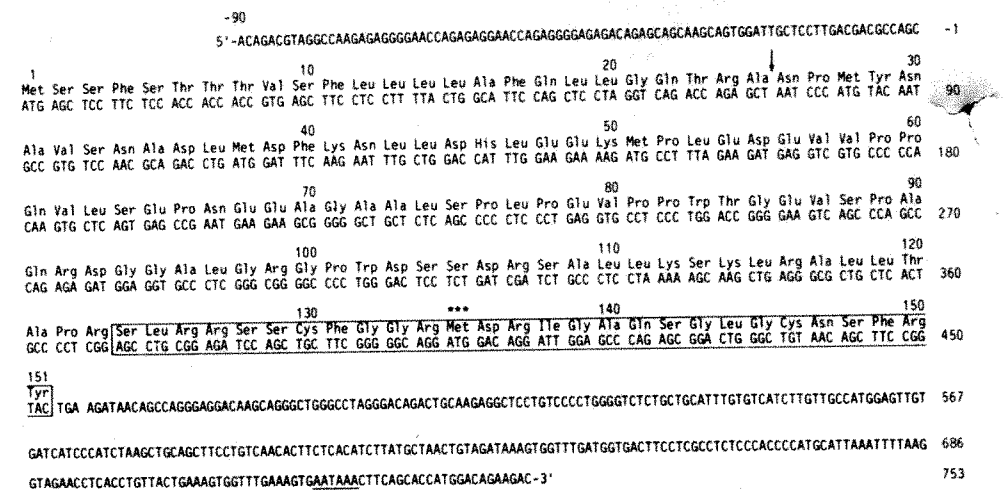


Fig. 2 Nucleotide sequence of plasmid phANP1 cDNA insert and deduced amino acid sequence of the precursor for α-hANP. The nucleotide sequence of phANP1 and phANP82 cDNA inserts were determined as described in Fig. 1 legend. Both sequences coincided except for 99 5′-terminal nucleotides shown in the figure which were missing from the phANP82 insert. Nucleotide residues are numbered in the 5′ to 3′ direction, beginning with the first residue of ATG triplet encoding the initiator methionine. Nucleotides on the 5′ side of residue 1 are indicated by negative numbers. The predicted amino acid sequence of the precursor for α-hANP is displayed above the nucleotide sequence, and its amino acid residues numbered beginning with the initiator methionine. Boxed sequence is of α-hANP. The arrow indicates the most likely processing site for signal peptidase. The hexanucleotide which precedes the site of polyadenylation in many eukaryotic mRNAs[11] is underlined.

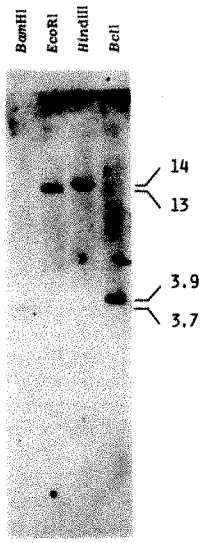


Fig. 3 Hybridization of human genomic DNA with a ^{32}P-labelled cDNA probe from plasmid phANP82. High-molecular-weight human DNA (8 μg) isolated from peripheral blood lymphocytes of several individuals by the procedure of Gross-Bellard *et al.*[24], using pronase instead of proteinase K, was digested to completion with 40 U each of various restriction endonucleases, electrophoresed on 1.0% agarose gel and blotted to a nitrocellulose filter[25]. The ^{32}P-labelled DNA probe was prepared from a 608-bp *Pst*I fragment (nucleotides 10–588 plus 29 G residues extended from nucleotide 10 of Fig. 2) of the cDNA insert of phANP82 as described previously[26]. 1.4×10^7 c.p.m. of the probe were hybridized with the filter for 16 h at 65 °C and then washed as described[27]. Numbers on the right of the figure indicate the size of the hybridized DNA fragment in kilobase pairs.

kephalin A[15] (Met-Ala-Arg-Phe-Leu-Thr-Leu-Cys-Thr-Trp-Leu-Leu-Leu-Leu-Gly-Pro-Gly-Leu-Leu-Ala-Thr-Val-Arg-Ala, where indicated amino acid residues are underlined) was noted.

The direct connection of γ-hANP to the putative signal sequence suggests that removal of the signal peptide and disulphide bond formation may yield natriuretically active γ-hANP. Also, the location of α-hANP at the carboxyl end of γ-hANP suggests that further processing takes place after the sequence Pro-Arg (residues 122–123). Although the Pro-Arg sequence has not been regarded as a typical processing signal in the synthesis of many peptide hormones, gastrin-releasing peptide is known to be processed at a Pro-Arg sequence to yield its C-terminal decapeptide amide in the mammalian intestine[16] and spinal cord[17]. α-hANP could be similarly processed out of the precursor.

The existence of multiple peptides with natriuretic activity in human atria led us to investigate (as described in Fig. 3 legend) whether the other related hANP genes are present in the human genome. Endonucleases *Bam*HI, *Eco*RI and *Hin*dIII do not have cleavage sites in the cDNA, but *Bcl*I has a cleavage site near the *Pst*I site (Fig. 1). The fragment to the right of the *Bcl*I site would not be expected form a stable hybrid with the probe used because the complementary sequence region is only 21 nucleotides long. A single hybridizing DNA fragment can be seen in each lane of the gel (Fig. 3), which strongly suggests the existence of a single gene for hANP precursor in the human genome, although the presence of multiple genes comprising a repetition of the same sequence cannot be excluded.

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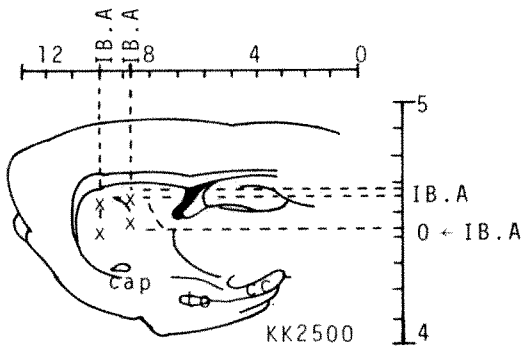
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Astrophysical consequences of n–n̄ oscillations

SIVARAM and KRISHAN[1] have suggested that the anomalously high flux of low-energy antiprotons observed in cosmic rays[2] is produced when free neutrons, which are possibly ejected in supernova explosions, undergo neutron–antineutron (n–n̄) oscillations as expected in some grand and partially unified theories[3]. Such oscillations are strongly suppressed in an external magnetic field[3–6] but by choosing the interstellar field strength, B, to be as low as 10^{-7} G and an oscillation time, $\tau_{n-\bar{n}} = 10^5$ s, these authors obtain[1], $\bar{n}/n \approx 10^{-4}$. Because n̄ and n β-decay to p̄ and p respectively, this implies $\bar{p}/p \leq 10^{-4}$, which compares with the observed value[2], $\bar{p}/p = (2.2 \pm 0.6) \times 10^{-4}$ at ~130–370 MeV/n. (Contrary to their[1] remark, the ratio p̄/p cannot exceed the ratio n̄/n, regardless of the number of neutrons ejected per proton in a supernova.)

However, before this suggestion, old limits on the stability of nuclear matter had been used to infer[4–6] $\tau_{n-\bar{n}} > 10^6$–5×10^7 s. Also, direct observations of free neutron beams had yielded the limit[7], $\tau_{n-\bar{n}} > 1.2 \times 10^5$ s, and this has been recently improved to[8] $\tau_{n-\bar{n}} > 10^6$ s. Furthermore, the interstellar magnetic field strength must be $> 10^{-6}$ G to account for observations of pulsar signals[9] and the galactic synchrotron background[10]. (We disagree that this value is ". . given by equipartition arguments . . (with) . . no physical basis . . "[1].) Thus, using these conservative limits, we obtain $\bar{n}/n \approx \bar{p}/p < 10^{-8}\ (\tau_{n-\bar{n}}/10^6\ \mathrm{s})^{-2}\ (B/10^{-6}\ \mathrm{G})^{-2}$. Moreover, this obtains at ~1 MeV/n, corresponding to the typical ejection velocity of $\sim 10^4$ km s^{-1} in a supernova. Thus the observation that $\bar{p}/p \sim 10^{-4}$ at ~200 MeV/n cannot be accounted for by this mechanism.

Note that even in the total absence of a magnetic field, neutrons would sooner β-decay than oscillate into antineutrons. This sets an absolute upper limit $\bar{n}/n < 10^{-6}\ (\tau_{n-\bar{n}}/10^6\ \mathrm{s})^{-2}$, thus making this process uninteresting in any conceivable astrophysical or cosmological context.

I thank Professor D. W. Sciama for discussions.

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SIVARAM AND KRISHAN REPLY—We have not stated anywhere in our paper[1] that all of the 10^{57} neutrons ejected by a single supernova are at an energy ~200 MeV, in which case there would indeed be an excess of energy involved. The only claim made is that here is a possible mechanism for directly producing p̄ at low (MeV) energies without any need for deceleration from energies of several GeV (with its attendant difficulties) inevitable in most models.

Second we felt that there was no need for Sarkar to have written down all the formulae for n–n̄ transition probabilities. These are well known and are the same formulae that we used. In fact for a $B = 10^{-7}$ G and $\tau_{n-\bar{n}}$ of 10^5 s, he also obtains $\bar{n}/n \sim 10^{-4}$. Regarding his chief point of contention that the strength of the interstellar magnetic field must be $\sim 10^{-6}$ G; we are surprised that Sarkar has overlooked the fact that in the latter half of our paper we have pointed out that if this field is assumed it would considerably lower $\bar{p}/p\ (< 10^{-6})$. (For 10^{-6} G, $\Gamma_B \gg \Gamma_{n-\bar{n}}$, and for 10^{-7} G, they are just equal.) We went on to point out that to build up the observed low-energy background of 10^{55} antiprotons in our Galaxy (as implied by the observed density of 10^{-4} eV cm^{-3}) we would require a few thousand explosions which would occur in periods $\sim 10^6$ yr which is the same order as the diffusion time for the produced particles to spread over the Galaxy and produce the observed background. More generally one can say that the number of antiprotons produced is

$$\bar{p} = N \left(\frac{\Gamma_{n-\bar{n}}}{g u_N B} \right)^2 f t, \qquad \Gamma_{n-\bar{n}} = \hbar / \tau_{n-\bar{n}}$$

where N is the number of neutrons per supernova, f the frequency of explosions and t the diffusion time $\sim 10^5$ yr. Thus if all low-energy antiprotons ($\bar{p} = 10^{55}$) in the Galaxy were produced by supernovae, then with $N = 10^{57}$, $f = 1/10$ yr, we would have an astrophysical constraint on the oscillation time $\tau_{n-\bar{n}}$. If $\tau_{n-\bar{n}} > 10^7$ s (as indirect evidence suggests), of course, the p̄ production would be too low. Measurements of p̄ should perhaps be made over a few MeV as this is the range where most supernova neutrons are produced.

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Formation of sapropels in the Tyro Basin

WE were most interested in the two companion papers pertaining to the recent formation of sapropels in the Tyro Basin, an isolated depression within the Hellenic Trench in the eastern Mediterranean Sea. Certainly the physiography within this trench system would be conducive to allowing localized near-bottom anoxia because of limited bottom water ventilation as related to outcrops of Messinian salt, as well pointed out by Jongsma *et al.*[1] and de Lange and ten Haven[2].

We are concerned, however, with their statement: "Assuming an undisturbed sedimentation and taking a relatively high sedimentation rate of 5 cm kyr^{-1}, we conclude that the recovered sediment column of 400 cm of solely sapropel to sapropelic sediment indicates a period of at least 80,000 yr."

Instead, we suggest that the 4-m long section was deposited in much less time. There is ample evidence of resedimentation events in similar isolated basins during the recent geological past and the present throughout the Hellenic Trench system[3–5], as well as on the Mediterranean Ridge immediately south of the trench[6,7]. In particular, redeposited sapropels have been identified in dated piston cores from these regions. Gravity-driven processes triggered by the active tectonism in the region have resulted in stratigraphically displaced and expanded-thickness sapropel sections[8,9], sometimes in excess of 4 m, and in dilution of organic matter content. As a result of this prevalent resedimentation process, cores from the trench show some of the highest sedimentation rates (to >400 kyr^{-1}) yet measured in the Mediterranean Sea[4]. Thus, their conclusion of a 400-cm long sapropelic section representing 80,000 yr of undisturbed sedimentation is unrealistic. It is equally plausible that at least part of the sapropelic section cored in the Tyro Basin is redeposited, and equivalent to the upper sapropel (S_1) dated at ~8,000 yr BP and mapped over much of the eastern Mediterranean.

L-DGO contribution no. 3653.

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TROELSTRA *ET AL.* REPLY—We agree with McCoy and Stanley's view that the age at the bottom of the Tyro Basin core must be considerably younger than we had assumed in our papers, which were mainly based on shipboard observations. Shore-based analyses, still in progress, have produced different data. Because of the different bottom water regimes in the Tyro and Kretheus basins, we would like to consider each basin separately.

First, we want to stress that, at present, conditions suitable for sapropel formation, namely an anoxic and reducing environment due to halocline water stratification, do exist in the Tyro basin. Laboratory studies on core P46 indicate that sediments were not deposited in exclusively pelagic conditions. The most important argument for this is the presence of fining-upward sequences in the core, indicating transport by mass-flows. Furthermore, faunal and floral composition through the core is rather uniform and suggests a relatively warm climate. If the age at the bottom of the core were 80,000 yr, a fluctuating faunal and floral pattern would be expected, due to climatic changes. Moreover, the presence at the bottom of the core of well-preserved algae, one species still in possession of bright green-coloured chlorophyll, indicates that little or no diagenesis took place. This is contrary to what one would expect if the sediments were deposited at 80,000 yr BP.

In contrast to the Tyro Basin, bottom waters in the Kretheus Basin are well-ventilated at present. Core P45 consists of alternate dark-coloured (? resedimentated sapropel) and lighter-coloured sediments. The latter contain abundant sand-sized grains of metamorphic rock indicating that sedimentation in the Kretheus Basin is also governed by mass-flow transport. Planktonic foraminiferal composition through the core is comparable with that in core P46.

We conclude that sedimentation rates in both basins must be high. At this stage, pending ^{14}C analyses, an entire Holocene age for both cores cannot be excluded. Resedimented S1 may be one of the major contributors to the sapropelic intervals in the cores, as correctly assumed by McCoy and Stanley.

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Sequence similarity among retroviruses—erratum*

RECENTLY Toh *et al.*[1] reported the existence of amino acid sequence similarity between the reverse transcriptase of certain retroviruses and the DNA polymerases of cauliflower mosaic virus (CaMV) as well as a region of hepatitis B virus (HBV) that probably encodes a DNA polymerase. For completeness, we draw attention to other regions of protein similarity between retroviruses (human T-cell leukaemia virus, HTLV; Moloney murine leukaemia virus, Mo-MuLV; and Rous sarcoma virus, RSV), HBV and CaMV that were not reported by Toh *et al.*

The sequences are presented in Table 1 and the relative positions of the similar regions indicated in Fig. 1. The common set of amino acids discussed by Toh *et al.* is conserved in the amino-terminal region of all retrovirus reverse transcriptases studied (regions I–III). These common sequences are located near the amino-terminus of the HBV protein and the centre of the CaMV polymerase. Another region of similarity, IV, not discussed by Toh *et al.*, is also found in all retroviruses and is present in the CaMV protein. Region V, reported by Toh *et al.*, to be present in Mo-MuLV and RSV, is also found on HTLV. We also draw attention to sets of amino acids that are conserved with respect to sequence and relative position between the reverse transcriptases of HTLV and Mo-MuLV (M1–M4) and HTLV and RSV (R1–R4).

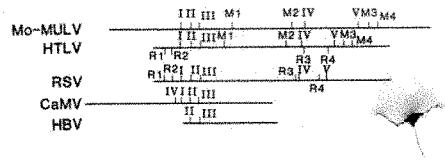
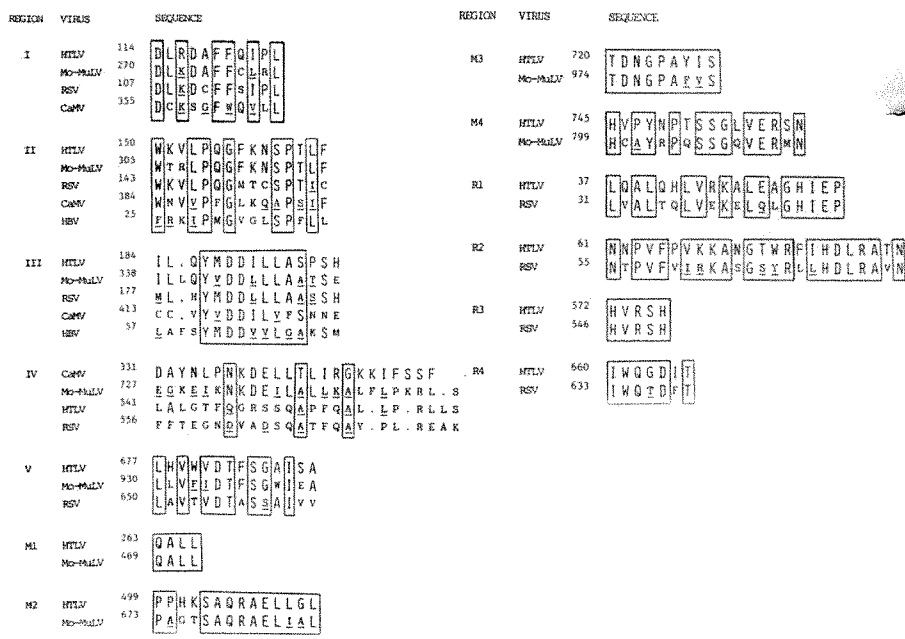


Fig. 1 Alignment of the polymerase gene products among five different viruses, depicting the regions shown in Table 1.

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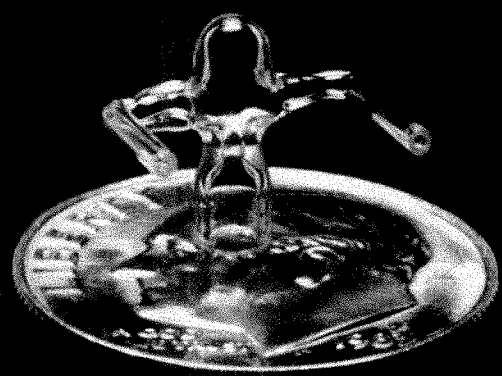
Table 1 Alignment of the similar regions in polymerase gene products among different viruses



Molony murine leukaemia virus, Mo-MuLV[2]; human T-cell leukaemia virus, HTLV[3]; Rous sarcoma virus, RSV[4]; cauliflower mosaic virus, CaMV[5]; and hepatitis B virus, HBV[6]. Common amino acids are boxed and conservative substitutions with respect to HTLV are underlined. For each sequence, the first amino acid is numbered.

* This paper was published in the issue of 17 May without title and Fig. 1 due to printing errors.

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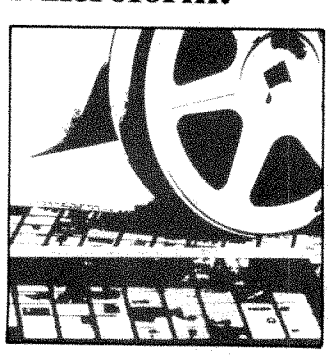
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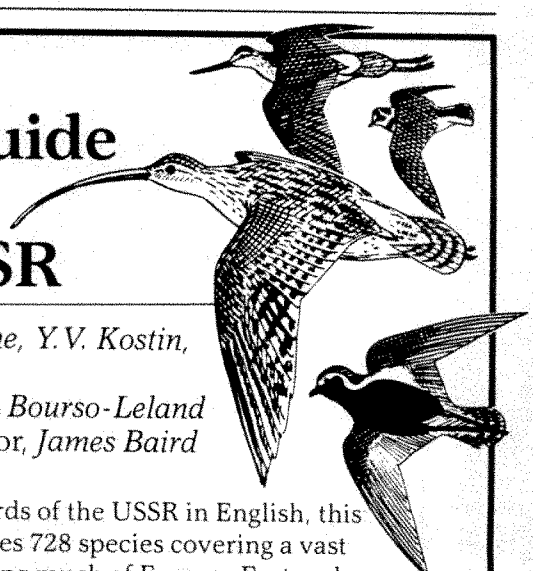
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The welfare stake

L.G. Goodwin

Of Mice, Models, and Men: A Critical Evaluation of Animal Research.
By Andrew N. Rowan.
State University of New York Press: 1984. Pp.323. Hbk $34.50; pbk $12.95.
Man and Mouse: Animals in Medical Research.
By William Paton.
Oxford University Press: 1984. Pp.192. Hbk £9.95; pbk £2.95.

AS Andrew Rowan says on p.31 of his book, "research on animals touches a very raw nerve in some people". Those who protest do so from diverse motives — they may identify with the helplessness of the animal or argue against "speciesism" as against sexism, racism and slavery. Some fight research they consider to be wasteful and frivolous, while others fear scientific progress of any kind. In recent years activist movements (reflecting the growth of violence in other spheres) have organized angry, destructive demonstrations; research animals are "liberated" and

> terrorist groups break into laboratories and use letter bombs to intimidate those who dare to remind the public of the advances in curative medicine and in public health that have followed from experiments on living animals [Sir Andrew Huxley in the foreword to *Man and Mouse*].

Yet, at the same time, there is a more intelligent, growing public concern about the way in which animals are used in laboratories. Although there is need for debate, biomedical scientists have sometimes been unwilling to talk.

The appearance of two excellent, outspoken books, one from each side of the Atlantic, is well timed. Andrew Rowan from Tufts University School of Veterinary Medicine, has a clear appreciation of the value and necessity of animal experiments but for long has waged a campaign for animal welfare, for the introduction of effective United States Government legislation and for a reduction in the numbers of animals used in what he considers to be wasteful or unnecessarily painful experiments.

Rowan feels that "the old platitudes and the arrogant cliché that doctor knows best are no longer sufficient in the face of a more sophisticated approach by the animal welfare movement". He deals with the history of animal research in the United States and with the attitudes and assumptions it evoked, lists the numbers of animals used, attempts to assess the pain and suffering caused, and discusses the "rights" of animals. He thinks that too many animals are used needlessly in education at all levels and deplores the "science fairs" that lead to unnecessary suffering of animals at the hands of high-school students. He also criticizes the work of the primate centres, various aspects of behavioural, psychological and other studies, and gives examples to support his belief that biomedical knowledge is being advanced at too great a cost in animal suffering. He believes that there is an opportunity for a great reduction in the wastage of animals in toxicological tests and for the revision of the requirements of the statutory bodies that demand them.

Sir William Paton, of the Department of Pharmacology in Oxford, has written a thoughtful, philosophical book that covers much of the same ground but from a different viewpoint. His objective is to make a strong case for *responsible* experimentation on live animals, and to look to the future. While there has been legislation in Britain on the conduct of animal experiments for more than 100 years, the Act of Parliament of 1876 is in course of revision and there is a European Convention on the use of animals in research.

In a reflective discussion of the ethical questions, Paton finds that the characteristic that gives human life a special value, different in quality from the lives of other animals, is the capacity to accumulate experience by the spoken, written and printed word and all that flows from it — the capacity to consent, to ask moral questions, to feel moral obligations and to feel responsible for animals. He finds no basis for claims that animals have "rights" (a human social concept linked with duties), but distinguishes between humans as moral *agents* and animals as moral *objects*, in respect of which humans accept duties. "Animal rights" may be replaced, with some gain, by the "moral worth of animal life".

Paton shows how, from the earliest times, accumulated knowledge from animal experiments (deliberate attempts to discover what was not yet known) has yielded great benefits for human and animal health. Many of these benefits were derived from work undertaken to acquire basic knowledge, with no particular benefit in view. Many problems still remain to be solved, especially in the control of diseases of impoverished, developing countries, and Paton warns that unreasonable harrassment and restriction of the efforts to acquire new knowledge is likely to inhibit advance and cause unnecessary human and animal suffering. Of all forms of animal suffering — and the "natural" life of animals in the wild is full of stress and tragedy — only that incurred by experiment, and the medical and veterinary practice based on it, offers the prospect of reducing further suffering and misery.

Both authors agree that the problem is to strike a balance and that Russell and Burch's "3 Rs" (put forward in *The Principles of Humane Experimental Technique*; Methuen, 1959) should be applied to all animals in experimental work — *replacement* by alternatives when possible, *reduction* in numbers and *refinement* of method. To these Paton adds a fourth, *responsibility* of the experimenter, a concern shared with the world outside the laboratory. Rowan and Paton agree that deliberate funding of research into methods for the relief of pain in animal experiments would be of advantage, and Rowan would like to see much more money available for the study of alternatives to animals. They agree that the LD_{50} test (originally designed for the accurate assay, against a standard, of the toxicity of agents such as diphtheria toxin in the preparation of vaccines) has been misapplied to substances for which an approximate measure of toxicity, using fewer animals and causing less suffering, would be more appropriate. They agree also that the statutory requirements laid down by official bodies for testing drugs and other chemicals should not be allowed to go unchallenged, but that tests should be discontinued when superseded by methods using less animals, causing less suffering or dispensing with animals altogether.

The paradox is that, along with public concern with the plight of experimental animals, there is increasing demand for drugs, foods and household products to be "safe". Safety testing, for a long time to come, will involve the use of animals. Television and radio programmes often report exciting advances in medicine, surgery and biology based on animal experiments without drawing attention to the essential part of such experiments, together with a rational mixture of non-animal and human experiment, in achieving the advance. Sensational accusations hit the headlines of popular newspapers which seldom give coverage when such accusations have been shown to be without foundation. The truth, it seems, is not news.

The debate will continue. As Sir Peter Medawar says:

> In the meantime we must grapple with the paradox that nothing but research on animals will provide us with the knowledge that will make it possible for us, one day, to dispense with the use of them altogether [*The Hope of Progress*; Methuen, 1972].

That debate will be all the better informed if the various protagonists read both of these books. □

L.G. Goodwin was formerly Director of Science at the Zoological Society of London. He serves on a Committee of the Royal Society responsible for drawing up guidelines for the use of animals in research.

Honeyed history

John B. Free

The Archaeology of Beekeeping.
By Eva Crane.
Duckworth/Cornell University Press: 1984. Pp.360. £25, $39.50.

THE history of man's relationship with the bee is a long and fascinating one; throughout the ages artists and poets have expressed appreciation of the bounty of the hive and the supposed virtue and selfless toil of the bee.

On the dust cover, the publisher claims that this is the first book "to explore the rich heritage of beekeeping archaeology". This is not true. However, earlier books in English that dealt exclusively with the history of beekeeping — among them Hilda M. Ransome's classic *The Sacred Bee in Ancient Times and Folklore* (of which a reprint is long overdue) and two works by H. Malcom Fraser, *Beekeeping in Antiquity* and *History of Beekeeping in Britain* — are no longer in print. During the past few years several other books have also included historical accounts of beekeeping. Nevertheless Dr Crane's book is a welcome and authoritative addition to the subject. Indeed it ranges more widely than its title implies and could more appropriately be called *A History of Beekeeping.*

Dr Crane provides detailed information on the latest archaeological and anthropological discoveries, notable among which are rock paintings depicting bee nests and honey-hunting in Zimbabwe, South Africa, Australia and central India. These and the long-known Spanish rock paintings are considered in relation to the honey-hunting techniques, still practised by primitive peoples in many parts of the world, to rob wild honeybee colonies of their honey and brood.

From Egyptian tomb paintings, ranging from 2400 to 600 BC, we obtain our first evidence of bees being kept in hives and of the harvesting and packing of honey into pottery vessels for later consumption. Horizontal stacked hives as depicted in the paintings are still to be seen today in Arab villages. Only a few Greek pottery hives and no Roman ones have been excavated; fortunately, however, Classical writers have left us with a wealth of information on their understanding of the honeybee colony and the types of beekeeping they practised.

During the past 2,000 years there has been a proliferation of hive types and methods of beekeeping, each adapted to local conditions. Illustrations and descriptions are given of clay pots, hollow logs, and wicker and straw baskets that are still used as hives in many parts of the world, and were undoubtedly used more widely in the past. Knowledge about primitive hives and beekeeping methods is not merely of scholarly interest, as their use in modified form can be helpful in encouraging beekeeping in developing countries where there is often considerable potential for honey production. So it is crucial that we retain such information, and Dr Crane's book could be most helpful in furthering this end. For example, the evolution of hives with movable combs can be traced back to a Greek basket hive, which here deservedly receives considerable attention. The principles it embodies form the basis of a hive now used in East Africa and elsewhere.

Because most hives are of perishable material, few old ones have survived. However more durable material was used to form the shelters in which many of the early hives were placed. Some were kept in beehouses, each with its own access to the exterior; others in open-fronted shelters, and, most commonly in the United Kingdom, hives were kept individually in recesses — bee boles — in stone or brick walls. Bee boles were most common in the eighteenth and nineteenth centuries, and the author obviously finds them particularly fascinating. She devotes 46 pages to discussion of bee boles and 33 pages to other hive shelters; the International Bee Research Association's records of the bee boles and shelters in the United Kingdom are given in an 80-page Appendix. While it is useful to have this information brought together in published form, it occupies nearly half of the book and so unbalances the presentation somewhat and gives it a bias towards the United Kingdom.

The book is attractively produced, contains much interesting and well-documented information, and many unusual illustrations. It will be a most worthy and useful addition to the beekeeping sections of libraries □

John B. Free is a Senior Principal Scientific Officer at Rothamsted Experimental Station, Harpenden, Hertfordshire.

Hope for an answer

William H. McNeill

Weapons and Hope.
By Freeman Dyson.
Harper & Row: 1984. Pp.340. $17.26, £10.95.

FREEMAN DYSON, whose last book set out to disturb the Universe, addresses this one to a more modest target: ". . . the gravest problem now facing mankind, the problem of nuclear weapons from a human rather than a technical point of view" (p.3). A human point of view means, above all, a moral point of view. But it is the morality of Odysseus rather than of Achilles, that is, of the cunning survivor, not the dead hero.

The author wanders back and forth between personal reminiscence, literary discourse and strategic discussion in a delightful if sometimes confusing way for a reader who seeks a rigorous argument about how to escape the threat of nuclear annihilation. Perhaps Dyson chose this approach to emphasize the human dimension. More likely it was because he recognizes logical weaknesses in his recipe for escape from our dilemma, but wishes to insist that hope — persistent and unremitting hope — is the really important ingredient since only so can cheap despair, paralysis of will and atrophy of moral sense be forestalled.

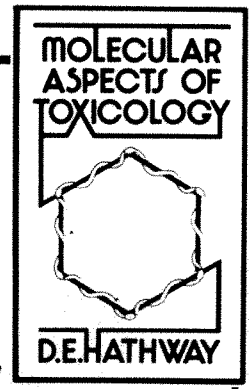
Dyson wants a world without nuclear warheads. He asserts that with the appropriate exercise of moral indignation, political negotiation and technical investment in non-nuclear defensive systems the dismantling of the last nuclear device might be achieved after fifty years — with luck along the way. To reach this goal, American and Russian soldiers must be convinced that nuclear arms are counter-productive — unusable in war, and debilitating to the morale of the armed services in peace. Only cooperation between what he calls "warriors" and "victims" can lead to the abolition of nuclear arms. The problem, then, is to find a concept of national defence that does not depend on nuclear weapons, and then attract a robust political following in support of the military policies such a concept implies.

Dyson discovers this in what he calls "live and let live", the principle that "We maintain the ability to damage you as badly as you can damage us, but we prefer our own protection to your destruction" (p.274). This rationale, Dyson thinks, would allow the United States to negotiate with the Russians for mutual reduction of existing nuclear weaponry all the way down to zero — perhaps. Holding to the idea of mutually assured destruction (plus planning on first use of nuclear tactical weapons in case of a Soviet attack in Europe), he says, will permanently prevent successful negotiation for the simple reason that Russian doctrine concerning the use of nuclear weapons differs from that of the United States, being aimed at survival against strategic rocketry, if need be by means of counterforce first strike.

I have difficulty in imagining just how Dyson's concept of "live and let live" could be implemented. His most surprising suggestion pertains to how American strategic rockets should be targeted:

> The answer to this question is simple: We should not aim them at all. Live and let live excludes assured destruction targeting as inconsistent with the basic principle that live Americans are to be preferred to dead Russians. Limited nuclear war and counterforce targeting are excluded because they are crisis unstable, especially since we are assuming no change in the Soviet practice of counterforce targeting. What then are we to do with our strategic weapons? We must simply maintain them as best we can, making sure that they are as invulnerable as

NEW BOOK for Toxicologists



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By D.E. Hathway

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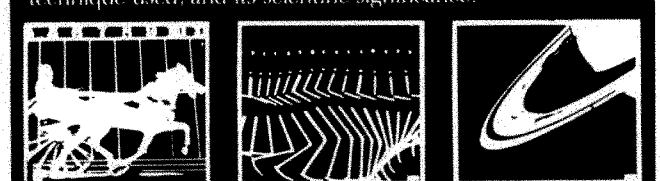
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possible to Soviet attack and that they are not aimed at anything in particular. This is what it means to say that we value them as bargaining chips but not as usable military weapons. [pp.275–276].

But aimed at nothing in particular is still aimed at something — what?



Courtesy of Harper & Row.

Freeman Dyson — morality of Odysseus.

Technological development does alter war plans and can divert arms races into unforeseen paths. For example, miniaturization of hydrogen warheads drastically altered weaponry and war strategy in the 1950s and 1960s, as Dyson lucidly explains. Similar shifts in thinking may well arise in the future. New defensive weapons may make our ICBMs as obsolete as, for example, the disappearing-carriage coast artillery guns built so proudly about a century ago. But it is hard for me to imagine that new weapons will not be as destructive as those they displace. Persuading sovereign states and their military experts to substitute weaker weapons for those they have runs counter to the choices that have always been made in times past. Can the concept of "live and let live" really reverse that drift? Or is there only hope?

What I find odd about Dyson's approach is his acceptance of the existing state system as unalterable. Relocation of sovereignty is how other arms races have ended. If ours is ever to do so that seems a far more probable scenario than anything Dyson proposes. The fact that peace through subjugation is unpalatable may be why Freeman Dyson rejects this possibility without considering it. He seems to feel that it would be treason to his given name. But would it? Perhaps he should supplement Homer with Vergil, to see how empires may also find patriots and loyal subjects, profoundly grateful for the peace they bring. □

William H. McNeill is Professor of History at the University of Chicago.

Probable ideas

Cyril Domb

Statistical Physics and the Atomic Theory of Matter, from Boyle and Newton to Landau and Onsager.
By Stephen G. Brush.
Princeton University Press: 1983. Pp.356. Hbk $45, £38.70; pbk $14.50, £12.70.

ELLIOTT Montroll, who died last December, was a leading figure in statistical physics for four decades. In a lecture delivered in 1958 entitled "One Hundred Years of Statistical Mechanics", he argued that the field was initiated by the 1857 paper of Rudolf Clausius on "The Kind of Motion We Call Heat". Admittedly there had been a good deal of activity since Newton, but activity is not the same as progress.

Montroll's thesis had the powerful backing of Willard Gibbs, who wrote in an obituary tribute to Clausius:

> The origin of the kinetic theory of gases is lost in remote antiquity and its completion the most sanguine cannot hope to see. But a single generation has seen it advance from the stage of vague surmises to an extensive and well established body of doctrine. This is mainly the work of three men, Clausius, Maxwell, and Boltzmann of whom Clausius was the earliest in the field and has been called by Maxwell the principal founder of the science. We may regard his 1857 paper as marking his definite entry into this field, although many points were incidentally discussed in earlier papers.

Stephen Brush argues that it is the task of the historian of science to record activity as well as progress, to make sure that false starts and dead ends are given proper coverage as well as the dramatic and exciting successes. Therefore, in addition to describing the fundamental contributions of Clausius, Maxwell, Boltzmann and Gibbs, who laid the foundations of modern statistical physics, he also tells us in detail of the struggles of earlier pioneers such as Boyle, Newton, and Daniel Bernoulli, and of the unsuccessful wave theory of heat which was an attempt to emulate the triumphant wave theory of light.

Maxwell was the first to appreciate that the language of probability and statistics is essential in relating the behaviour of a macroscopic body to the properties of its microscopic constituents. Boltzmann developed and extended Maxwell's ideas and introduced his famous hypothesis relating the entropy of a macrostate to the combinatorial weight of microstates. He also pursued the kinetics of the approach to equilibrium, and was forced to grapple with the irreversibility paradox — how it is possible for equations of motion, which are reversible on the microscopic scale, to give rise to irreversible macroscopic phenomena. The introduction of these novel ideas and the battle to get them accepted are among the most fascinating stories in the history of physics. Brush considers that these concepts may have helped significantly to bring about the acceptance of probability and indeterminancy in twentieth-century quantum physics.

Statistical mechanics played a central role in leading Planck to his famous quantum hypothesis. However Brush claims that the standard textbook account that the quantum of energy $h\nu$ was introduced to overcome the "ultra-violet catastrophe" is false and was invented by Paul Ehrenfest. Planck was looking for a formula which would fit the theoretical and experimental results on black body radiation. His result did not immediately produce much excitement in the world of physics. Gradually, as a result of Einstein's interpretation of the photoelectric effect, experimental developments in spectroscopy, and the success of Bohr's model of the atom in interpreting some of them, his new hypothesis gained acceptance.

A substantial part of the book is devoted to the history of modern fields of research, and here the author faces the difficult problem of coping with a burgeoning yet scattered literature. Many important results are first published in conference proceedings, and reliable guidance can be obtained only by consulting active researchers. Professor Brush has adopted this method, and has incidentally reproduced many interesting personal anecdotes.

The coverage given to different contemporary topics is subjective. The superfluidity of liquid helium receives exhaustive treatment, presumably because the author himself was actively engaged in the field. More space is devoted to this topic than to the whole of phase transitions and critical phenomena, each success or failure of the "collective excitation" approach of Landau and the rival "Bose Einstein condensation" of London and Tisza being faithfully recorded. By contrast the theory of superconductivity receives quite cursory treatment.

It is inevitable in a book covering such a wide variety of topics that there should be some omissions. For example, in the area of phase transitions (with which I myself have been concerned) the treatment of the solid–liquid transition makes no reference to the important work of Sir Francis Simon and his collaborators on the melting curve at high pressures. The concepts of scaling and universality which play such an important part in the modern theory of critical phenomena are not given proper attention. There is no account of the return to Landau's ideas in their Landau–Ginzburg form for the development of the renormalization group. The work of Patashinskii and Pokrovski in the Soviet Union should also be mentioned. In the classical period no reference is given to the work of Ornstein and Zernicke who provided the first correct description of correlations near the critical point; and Andrews's Bakerian Lecture to the Royal Society in 1869 deserves to be included in the

reference list. All these points might perhaps be taken into account in a second edition.

One of the tasks of the historian of science is to see that credit is properly assigned for scientific discoveries. Professor Brush emphasizes the part played by Wilhelm Lenz, the supervisor of Ernst Ising, in formulating the Ising model; in fact Brush uses the terminology "Lenz–Ising model". He also tells the sad story of J.J. Waterston who anticipated some of the ideas of Clausius in 1845. Waterston's paper was rejected by the Royal Society and the manuscript was not even returned to the author. In addition, the respective contributions of Bose and Einstein to the development of Bose–Einstein statistics, and of Fermi and Dirac to Fermi–Dirac statistics are described.

A useful feature of the book is the wide and detailed reference list, and research scientists will be surprised to find how the literature on the history of science has grown. For example, I learnt of the existence of English translations of several of Clausius's fundamental papers, and of a publication of Waterston's collected scientific papers. In all, this is a book from which all who are interested in statistical mechanics can benefit. □

Cyril Domb is Professor of Physics at Bar-Ilan University, Ramat-Gan, Israel.

Managing disease

J.W. Deacon

The Nature and Practice of Biological Control of Plant Pathogens.
By R. James Cook and Kenneth F. Baker.
American Phytopathological Society: 1983. Pp.539. $43.

BIOLOGICAL control of plant diseases has generated much interest in recent years, in large part because of the efforts of a few workers such as James Cook and Kenneth Baker. The achievements up to 1974 were summarized and discussed in their earlier book, *Biological Control of Plant Pathogens*; this new work is a companion volume rather than a replacement, special attention being given to developments in the past decade. The book is impressive: quite simply, there has never been a better exposition of the "ecological" approach to plant disease control. The authors draw upon their first-hand experience and wide knowledge, and the result is an authoritative and highly readable account of the subject. It should be welcomed by both specialists and university students in the plant sciences.

The title, however, is surely misleading. In the view of many people, biological control is brought about by a third party (i.e. not by the host or the pathogen), either applied directly or promoted by manipulating other factors. Instead, the authors have defined biological control so broadly that it leads to near-absurdities, for example when we are told (p.391) that the growing of genetically different crop cultivars in neighbouring areas is a form of biological control because it restricts the spread of pathogen races specific to any one cultivar. Further (p.407), the use of lime and inorganic fertilizers are said to be ways to achieve biological control if they affect plant resistance. The term then loses all useful meaning.

Semantics apart, this book is actually about *disease management* by manipulating the host, pathogen or environment (including microorganisms) to achieve what the authors term "sustainable agriculture". As such, it is timely and thought-provoking, though it could usefully have included chemical control methods, because a sustainable agriculture must harness, rationally, all available resources for plant protection. Let us hope that plant pathologists will follow the entomologists' lead in this respect.

Returning to biological control in a restricted sense, there are several successful techniques that are applied widely in commercial practice. But all too many of the "successes" discussed in this book have never reached the stage of exploitation, and the authors could have been more critical in this respect. For example, their earlier book cited astounding benefits when *Bacillus subtilis* was applied to seeds of crops in Australia: in field trials, carrot yields were increased by 48% and oat yields by 40%, even when the untreated crops showed no obvious disease symptoms. Baker was associated with this work, so now we could expect to learn of further developments. Instead, the earlier reports are merely repeated. Why aren't Australian growers using *B.subtilis* and getting huge yield increases? Was the work abandoned or were subsequent trials inconclusive? In order to justify its title, the book should have addressed such questions. And why cite yet more "successes" in the use of seed-applied microbes to control damping-off diseases? We knew this in the 1950s and there have been regular reports ever since, but the method has not been adopted commercially because we have cheap, dependable and reasonably safe fungicides that give the necessary short-term protection to seedlings.

In short, biological control will achieve more converts if it is *seen* to be approached with realism and less of an evangelical spirit. But this is a relatively minor point. Cook and Baker's new book will encourage and assist workers in this field, while helping to ensure that the momentum of work on biological control is sustained. □

J.W. Deacon is a Lecturer in Microbiology at the University of Edinburgh, currently working at the Citrus and Subtropical Fruit Research Institute, Nelspruit, South Africa.

Clearly intermediate mechanics

J.W. Humberston

Theoretical Mechanics.
By E. Neal Moore.
Wiley: 1983. Pp.456.
£28.10, $37.90.

THERE are already so many books on classical mechanics, that one might well question the need for yet another — the field is not growing in the sense that fundamentally new material is continually being added to it. *Theoretical Mechanics* deserves attention, however, because the style of presentation and the philosophy behind it are somewhat unusual.

The book is designed for students who are already familiar with the subject matter covered in the usual undergraduate course in basic mechanics and who wish to have an introduction to more advanced topics such as Hamiltonian and Lagrangian mechanics, variational methods and canonical transformations. The author believes that an initial understanding of these topics is more likely to be acquired by practice in solving problems than by studying the formal structure of the subject. Accordingly, he has provided numerous worked examples and sets of problems at the end of each chapter.

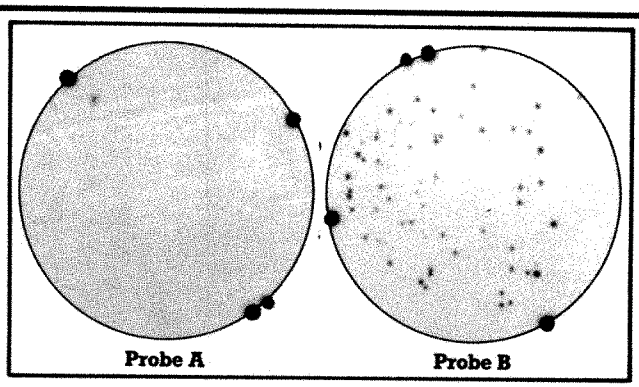
The book begins with a brief review of basic Newtonian mechanics, and, in addition to the topics already mentioned, contains chapters on central force motion, rotations, oscillations and stability, and a brief introduction to the special and general theories of relativity. Several of these topics are treated in more elementary books on mechanics, but the presentation here is usually more advanced; for example, the Runge – Lenz vector is introduced in the discussion of the motion of a particle under an inverse square law of force.

Eighty pages are devoted to several mathematical appendices, some of which are rather inappropriate in a book at this level. So while the appendices on tensors and group theory are useful, it hardly seems necessary to give the elementary properties of vector operations, determinants and matrices. Finally, there is a quite comprehensive bibliography and a list of references to articles on mechanics published in the *American Journal of Physics*.

The book is well produced, contains very few typographical errors and is written in a pleasantly informal style. But, as the author himself admits in the preface, rigour is often sacrificed in the interests of clarity. □

J.W. Humberston is a Lecturer in the Department of Physics and Astronomy at University College London.

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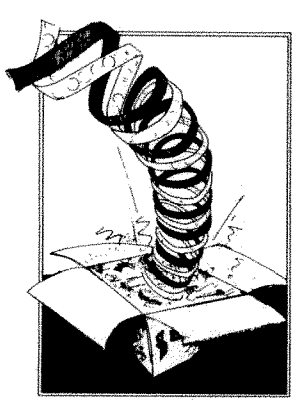
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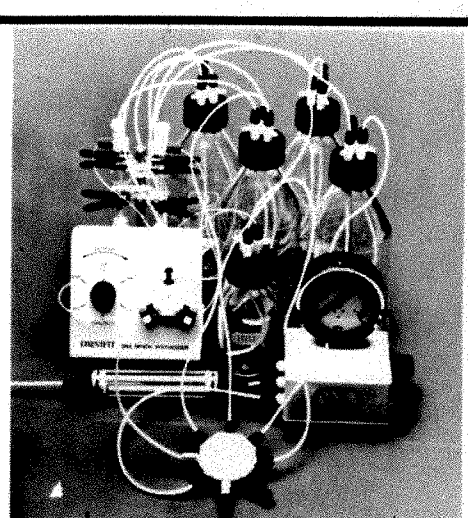
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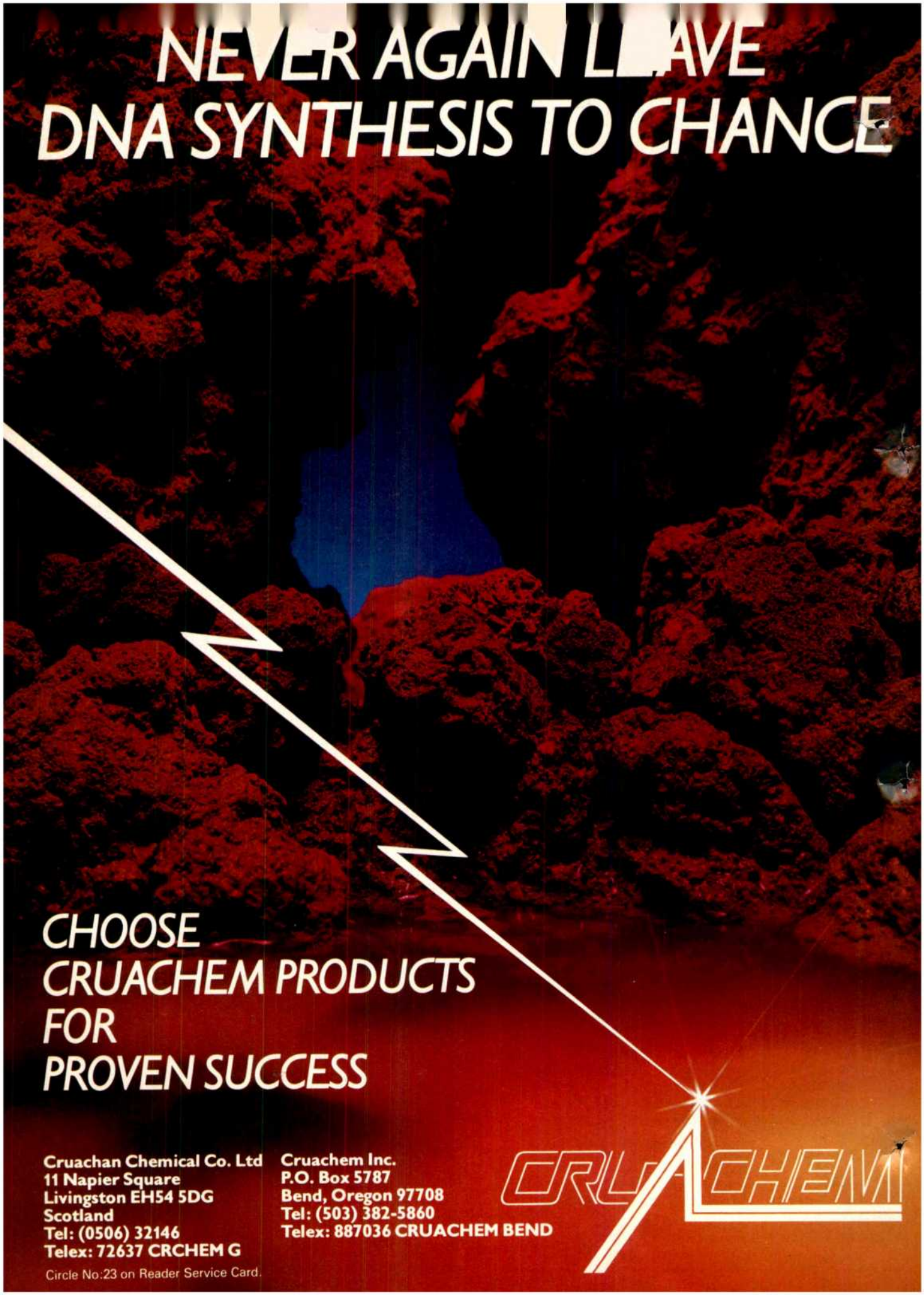
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● The Industrial Microbiology division of Celltech operates a custom synthesis service for oligonucleotides. A new price list has just been published and copies are available either through *Nature*'s Reader Enquiry Service or direct from Celltech.
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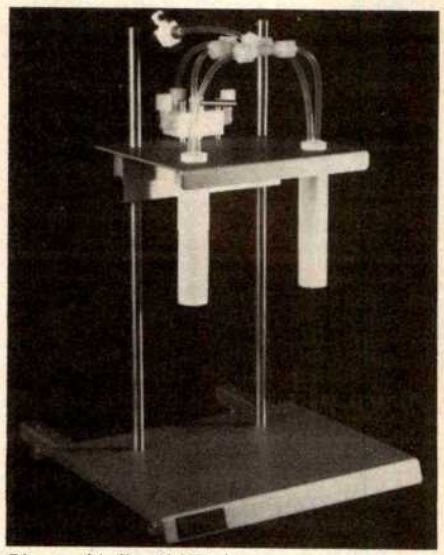
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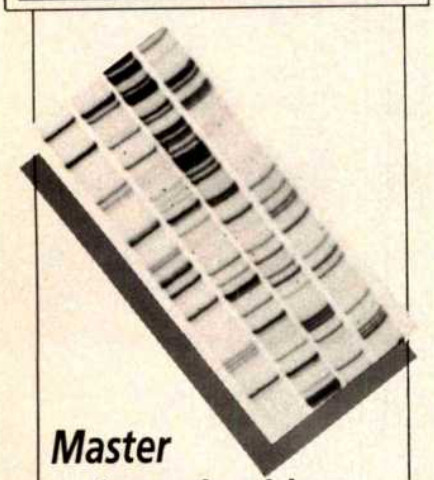
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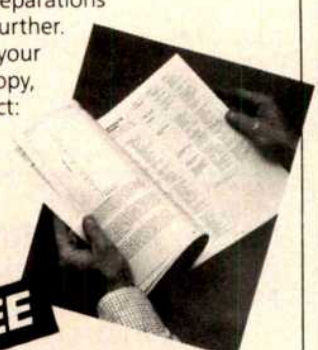
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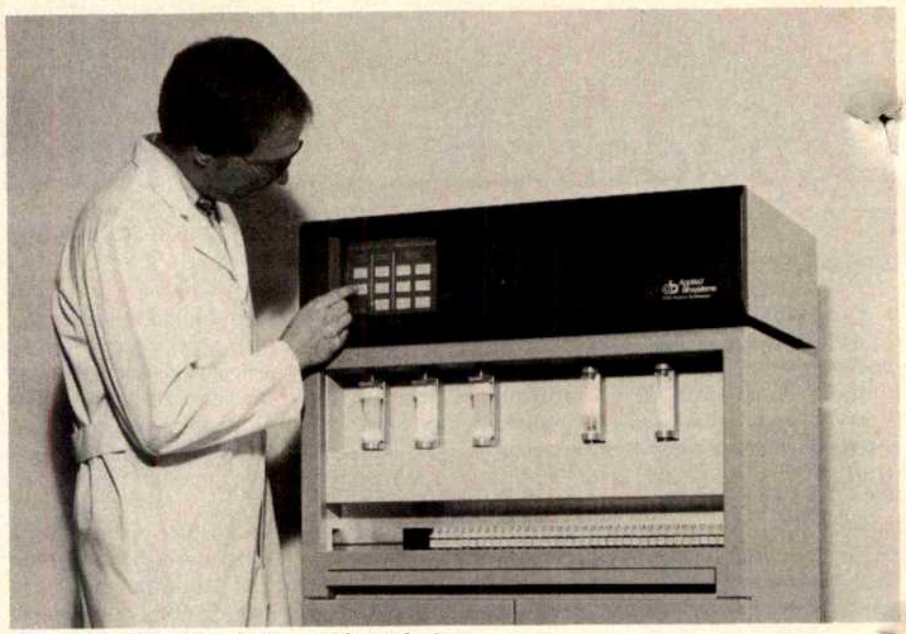


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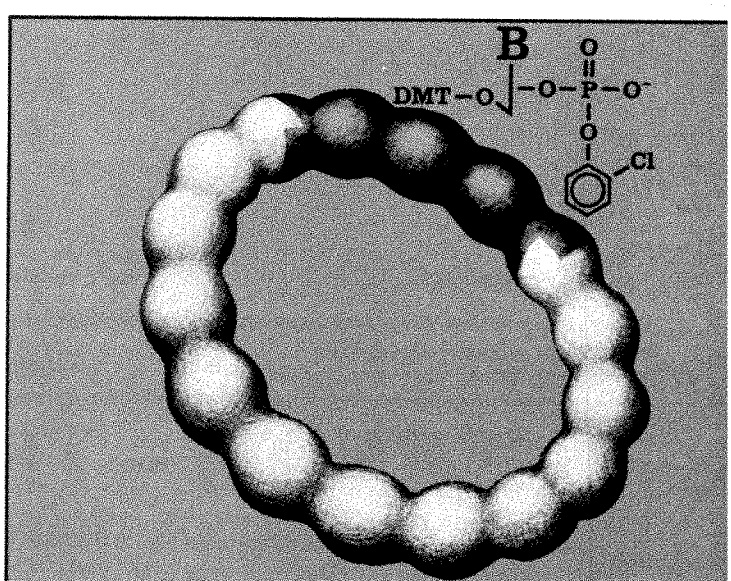
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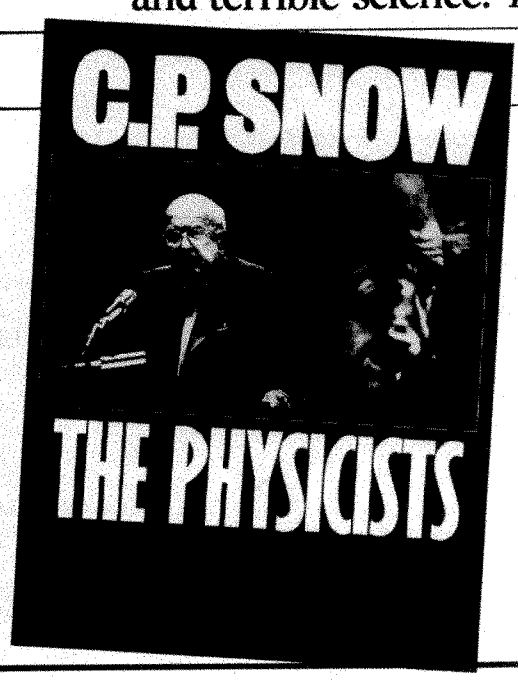
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(W1074)A

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The projects entail the development of simplified assay systems for factor VIII and the synthesis of novel affinity ligands and their exploitation in solid and liquid phase affinity process technologies for the large scale purification of human factor VIII. The posts are part of a 5 year programme of joint research with the Blood Products Laboratory, Elstree. Applicants should ideally have some experience in blood or protein fractionation although some training in clotting factor assays will be available at BPL.

Initial salaries will be £7,190 — £8,530 p.a. for the postdoctoral research associate and £5,880 p.a. for the graduate research assistant with annual increments according to National Scales 1A and 1B respectively.

Potential applicants should send a curriculum vitae detailing relevant experience and the names and addresses of two referees to Dr C R Lowe, The Biotechnology Centre, Downing Street, Cambridge CB2 3EF, UK by July 6th, 1984. Previous applicants need not apply.

(2960)A

Exciting research opportunity in West Germany; Partake in studies on intracellular metabolism and ion movements in organelles and intact cells

Ideal candidate must have a PhD (junior faculty) and should have experience in high resolution NMR technology (^{31}P, ^{23}Na) and its application to complex biological systems. A Bruker multinuclear NMR instrument (AM 400 widebore) is expected to be intstalled by the end of June 1984. In addition to the researcher's salary, this federally funded position includes salary for one technician, start-up funds and an appropriate supply budget. The research laboratories are part of the Max-Planck-Institut fuer Systemphysiologie in Dortmund, West Germany. Starting date: August, 1984.

Please send CV and references to Dr. Rolf Kinne, Director, Max-Planck-Institut fuer Systemphysiologie, Rheinlanddamm 201, 4600 Dortmund 1, West-Germany.

(W1047)A

NATIONAL INSTITUTE FOR HIGHER EDUCATION DUBLIN

Applications are invited for a:-

LECTURESHIP IN CHEMICAL SCIENCES

The School of Chemical Sciences is centrally involved in the BSc degree course in Analytical Science, in addition to servicing other courses, and possesses a comprehensive range of modern analytical instrumentation. The appointed candidate will become involved in the implementation and further development of teaching programmes and will be expected to initiate and develop programmes of research and to become involved in expanding links with industry.

Applicants should have interests in one or more of the following areas:

Analytical Science (instrumental aspects, inorganic analysis, separations science),

Applied Chemistry (areas of applied inorganic, organic or physical chemistry).

Applicants should be well-qualified academically with appropriate research, teaching or industrial experience.

Salary Scale: Lecturer IR£13,797 - IR£18,807
Assistant Lecturer IR£10,727 - IR£13,218

Application forms, and further details, are available from the Personnel Office, National Institute for Higher Education, Glasnevin, Dublin 9, Ireland. Closing date: 3 August 1984.

(2875)A

UNIVERSITY OF EAST ANGLIA
Norwich

SENIOR RESEARCH ASSOCIATE

Applications are invited for a one-year appointment in the Climatic Research Unit to work on a project investigating climatic change over the past century. The successful candidate will have considerable computing experience and a strong background in meterology and/or climatology. A doctorate in an appropriate discipline, a capability for innovative statistical analysis, and experience in report writing are required. The salary will be on the scale £7,190 to £11,615 per annum (under review).

A starting date no later than October 1st 1984 is desired. Applications should be sent, preferably by August 15th, to the Director, Climatic Research Unit, University of East Anglia, Norwich, NR4 7TJ, from whom further particulars of the appointment are available. (2962)A

UNIVERSITY OF STIRLING

CHAIR IN PSYCHOLOGY

Applications are invited from suitably qualified persons in Psychology and related disciplines without any restriction on the applicant's field of interest. Salary within the professorial range.

Further particulars are available from the Secretary, University of Stirling, Stirling FK9 4LA; Tel: 0786 73171, Ext 2250, to whom applications should be sent by 30 September 1984. (2953)A

M.R.C. Clinical Research Centre
(Northwick Park Hospital)
Watford Road,
Harrow, Middlesex HA1 3UJ.

Technician in Electron Microscopy

A vacancy exists for a Technician to work in the well equipped section of Electron Microscopy at the Clinical Research Centre. The successful applicant will be required to work on a variety of research projects, particularly involving the application of immunolabelling techniques, including low temperature embedding methods and immunogold labelling. Previous experience is required and the ability to carry out the general care of Philips 300 Microscopes would be an advantage.

Minimum qualifications are an HNC or science degree in an appropriate subject with a salary within the range £6,114-£8,370 depending on age and experience.

Application forms and further details may be obtained by writing to Miss J. Cottam, Personnel Section, Quoting ref 112/2/4133.

Closing date: 13th July, 1984.
(2935)A

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elf bio recherches — a subsidiary of Elf Aquitaine and Sanofi

HEAD PROTEIN PURIFICATION PILOT

Toulouse Labege Centre — Elf Bio Recherches, a specialist in biotechnology based at Toulouse Labege, its new research centre, is strengthening its research teams, and is recruiting its head of protein purification pilot plant. This person will be responsible for transferring laboratory protocols and developing pre-industrial purification systems in order to obtain products for pharmaceutical use. He will be responsible for a team of research scientists and technicians. The successful applicant will have been trained to a high level in biochemistry and chemical engineering (PhD or equivalent) and will be able to prove several years of experience in pre-industrial and/or industrial purification.

For this position, please write to Mr. Fabre, quoting the reference B/T2008N.

71 bis, allées Jean Jaurès —
31000 TOULOUSE — FRANCE
tel: 010 33 (61) 63.70.63.

(2976)A

RESEARCH POSTS IN

Medicinal Chemistry
Cellular Electrophysiology
Neuroimmunology
Neuropharmacology

Applications are invited for positions at Senior Scientist level, tenable from 1 January 1985.

The Institute will be housed in well-equipped and spacious new laboratory accommodation which is being built within the precincts of University College London, with which strong academic links will be forged. The scientific staff will be built up to 25–30 by 1987.

The Institute will embark on a programme of basic research in neurobiology, concentrating initially on mechanisms involved in nociception, with the long-term aim of developing new compounds of potential therapeutic value. Initially there will be six laboratory groups (Medicinal Chemistry, Biochemistry, Neuropharmacology, Cell Biology and Cell Culture, Immunology, Cellular Electrophysiology) which will collaborate on a range of individual projects.

Applicants for these positions should be 28–35 years old, with at least two years' post-doctoral experience. The research programme of the Institute will be interdisciplinary in its approach, and an ability to contribute original ideas and to collaborate effectively are particularly important.

Medicinal Chemistry

For this position a good knowledge of synthetic organic chemistry and an enthusiasm for active collaboration with biologists in the design and testing of new compounds are essential. Experience of working in the pharmaceutical industry would be an advantage.

Cellular Electrophysiology

This post will require the application of electrophysiological methods to the analysis of drug action at the cellular level. Candidates should have considerable experience of micro-electrode recording techniques or *in vitro* preparations, preferably with experience of patch-clamping.

Neuroimmunology

This post will entail the applications of immunological methods to the study of neurones in culture and *in situ*, as a means of investigating neuropeptides surface antigens and receptors. Previous experience in this area is less important than a strong background in biochemistry, cell biology and immunology, and an innovative approach to research.

Neuropharmacology

Expertise in electrophysiological recording methods *in vivo* and *in vitro* is required. The work will involve studying the effects of drugs on activity in specific sensory pathways in the peripheral and central nervous system.

Salary negotiable around £15,000 p.a. according to experience and assistance with relocation costs available if relevant.

Applications by 16 July 1984, including c.v. and names of two referees, to Professor H.P. Rang, FRS, Director, Sandoz Institute for Medical Research, Department of Pharmacology, University College London, Gower Street, London WC1E 6BT (Tel: 01-388 5314), from whom further information can be obtained.

(2898)A

SANDOZ

Department of Veterinary Pathology, University of Edinburgh and the Moredun Research Institute, Gilmerton Road, Edinburgh.

Applications are invited for the following appointments to an A.F.R.C. Linked Research Group between the Departments of Veterinary Pathology, University of Edinburgh and the Moredun Research Institute, Animal Diseases Research Association, Edinburgh. The appointments are for five years.

The research programme is concerned with fundamental studies of the sheep immune system in relation to disease mdoels of persistent viral infections of sheep. The posts are located within the University Department but there will be frequent interaction with the Institute as dictated by the research programme. The appointees will work with experienced research workers in immunology and virology.

Appointments:

Research Fellow 1A — Postdoctoral cellular immunologist for studies on the in vitro analysis of lymphocyte and accessory cell function and immune regulation in vivo within lymphoid tissue (Ref. LRG31/1). Salary £8,080 — £9,875.

Research Fellow 1A — Postdoctoral immunochemist/biochemist for biochemical characterisation of lymphocytes and accessory cell membrane glycoproteins, biochemical aspects of antigen presentation using viral polypeptides (Ref. LRG31/2). Salary £8,080 — £9,875.

Research Technician — (TG4/5) with experience in cellular immunology for studies on lymphoid cell function and monoclonal antibody work. Salary on range £5,826 — £6,898 (under review) with placement according to experience and qualifications. (Ref. LRG31/3).

Research Technician — (TG4/5) with experience of biochemistry for immunochemical work on cellular and viral antigens. Salary on range £5,826 — £6,898 (under review) with placement according to experience and qualifications. (Ref. LRG31/4).

Technical Assistant — (TG2) for general laboratory duties connected with the above research programme. Salary £4,662 — £5,151. (Ref. LRG31/5).

All appointments carry full superannuation and National Insurance benefits.

Applications (curriculum vitaes, 2 referees) for posts LRG31/1 and LRG31/2 should be sent to the Secretary to the University, Old College, South Bridge, Edinburgh, from whom further particulars can be obtained. Applications for posts LRG31/3, 4 and 5 should be sent to the Personnel Office, 63 South Bridge, Edinburgh. Informal enquiries can be made to Professor I. McConnell, Department of Veterinary Pathology, Royal (Dick) School of Veterinary Studies, Summerhall, Edinburgh. Tel 031-667 1011 ext 5322. Closing date for applications 6th July 1984. (2910)A

MAITRE DE CONFERENCE/ASSOCIATE PROFESSOR OF ANIMAL PHYSIOLOGY, UNIVERSITE DE PAU — FRANCE

PhD degree in nutrition or in sensory physiology required. Duties: teaching in French language of undergraduate general biology students; active participation in experimental ecology research: relations between legumes and animals (particularly insects) in a new CNRS-University Institute. Salary range of MC1 (88,560 FF) to MC3 (126,000 FF), will be on a 1-year renewable basis.

Application including curriculum vitae should be sent before 1st July 1984 to Pr. V. LABEYRIE, IBEAS, Université de Pau, Casino Municipal, 64000 PAU, France. (W1046)A

SUNDERLAND POLYTECHNIC

Faculty of Science
BIOLOGY DEPARTMENT

Research Assistant in Microbial Technology

Salary scale: £4,947-£5,649 (under review)

Applicants should hold a first or second class honours degree in a relevant biological science. The successful candidate will be expected to register for a higher degree and will work with a research group involving staff at Sunderland and Teesside Polytechnics and at Newcastle University. The project will be concerned with developing techniques for the immobilisation of carbon monoxide-oxidising bacteria.

Application forms and further details are available from the Personnel Officer, Sunderland Polytechnic, Langham Tower, Ryhope Road, Sunderland, SR2 7EE, or telephone (0783) 76231 Extn. 11.

Closing date: 11 July, 1984. (2941)A

FACULTY POSITION IN PHARMACOLOGY

Applications are invited for a tenure track appointment as an Assistant or Associate Professor in the Department of Pharmacology at the University of Tennessee Center for the Health Sciences. Applicants as Assistant Professor should possess the Ph.D., M.D., or other doctoral degree, have at least 2 years of postdoctoral experience, and have demonstrated ability to conduct an independent research program which applies modern constructs and methods of biochemistry, molecular biology, immunology, or genetics, to research problems in Neurosciences as applied to Pharmacology. Responsibilities include the development of an independent research program, and participation in the Pharmacology Department teaching programs.

Applicants as an Associate Professor should have 5 years of experience and an established externally supported research program. Please forward curriculum vitae, a research proposal, and arrange three letters of reference to be sent to Dr Murray Heimberg, Chairman, Department of Pharmacology, University of Tennessee Center for the Health Sciences, 874 Union Avenue, Memphis, TN 38163. Inquiries should be received prior to July 15, 1984, for a position available immediately. UTCHS is an equal opportunity/affirmative action employer. (NW624)A

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF PHYSIOLOGY + PHARMACOLOGY

TEMPORARY LECTURESHIP IN PHYSIOLOGY & PHARMACOLOGY

Tenable for three years from 1 October 1984. Preference will be given to candidates with an interest in the fields of muscle physiology, neurobiology or membrane physiology.

Salary at appropriate point within range £7,190 to £8,530 per annum (under review), plus USS.

Further particulars may be obtained from the Establishments Officer, The University, College Gate, St Andrews, Fife KY16 9AJ, to whom applications (two copies preferably in typescript) with the names of three referees, should be sent to arrive not later than 16 July 1984. (2968)A

LEICESTER POLYTECHNIC

SCHOOL OF CHEMISTRY

PHYSICAL CHEMISTRY LABORATORY TECHNICIAN

Scale 4/5 Salary
£6,264 — £7,896.

The postholder will be solely responsible for the day-to-day running of a Physical Chemistry teaching laboratory. The successful applicant will be likely to hold an HNC or equivalent qualification, have some experience of laboratory organisation and be competent in the routine maintenance of laboratory equipment. He or she would be expected to participate in the work of the laboratory and collaborate with other members of the technical and academic staff.

Further information may be obtained from Dr C Groves on ext 2368.

Application forms from the Personnel Office, Leicester Polytechnic, PO Box 143, Leicester LE1 9BH. Tel (0533) 551551 Ext. 2303. (2939)A

UNIVERSITY OF LEICESTER

DEPARTMENT OF PHYSIOLOGY

GRADUATE RESEARCH ASSISTANT

Ionic Channels in Muscle

Applications are invited for the above post to work with Dr N B Standen and Dr P R Stanfield on a 3 year project supported by the MRC involving patch-clamp studies of skeletal muscle fibres. In addition to single channel recording from adult and cultured cells, carried out on-line to a computer, methods for channel reconstitution and their study in lipid bilayers will be used. The successful applicant will be expected to register for a PhD degree. Commencing 1 October, 1984.

Salary on scale £6,310 — £7,190.

Applications including the names of two referees to Dr P R Stanfield, Department of Physiology, University of Leicester, Leicester LE1 7RH, who will be pleased to supply further information. Closing date, Monday 16 July, 1984. (2930)A

KING'S COLLEGE LONDON

DEPARTMENT OF PHARMACOLOGY

RESEARCH TECHNICIAN

Applications are invited for the above position to assist in a project concerning the modification of lipoproteins by arterial wall cells and its relationship to atherosclerosis. The post is for 3 years initially and is available immediately. It is funded by a Medical Research Council Grant awarded to Dr David S Leake.

Qualifications: BSc, HNC, or equivalent in Pharmacology, Biochemistry or related discipline. Experience in tissue culture would be an advantage, but not essential as training will be given.

Initial salary (according to experience) £6,146 pa or £6,401 pa (incl) rising to £6,689 pa (incl) (under review). 5 weeks annual holiday. Superannuation Scheme.

Apply in writing, with CV including 2 referees, to: The Assistant Secretary Personnel (ref: 249025) King's College, Strand, WC2R 2LS. (2931)A

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF PHARMACY

(PHARMACEUTICAL CHEMISTRY) UGC "New Blood" LECTURESHIP IN PHYTOCHEMISTRY

Applications are invited from candidates under 35 years of age who do not hold a tenured post in a UK university for a "New Blood" Lectureship in Phytochemistry. Candidates should hold a first degree in Pharmacy, Chemistry or relevant Biological Science and have completed a higher degree in some aspect of Phytochemistry. The successful candidate will be expected to participate in the present research activities of the Phytochemistry Group, to initiate novel research in Phytochemistry and to teach relevant chemical courses leading to BSc(Hons) Pharmacy and MSc degrees. Preference will be given to candidates with particular interests in structural elucidation, quantitative analysis or biological function of natural products.
Salary for the post will be on the Lecturer scale £7,190 — £14,125 per annum. USS benefit.
Further particulars and application forms (please quote ref: 34/84) are available from Staff Office, McCance Building, 16 Richmond Street, Glasgow G1 1XQ. Closing date for applications: 12 July 1984. (2925)A

UNIVERSITY OF LEICESTER

DEPARTMENT OF MICROBIOLOGY

RESEARCH TECHNICIAN

Applications are invited for a three-year MRC-funded appointment to commence 1st August 1984. The successful candidate will join a research group working on the pathogenicity of polio and related picornaviruses and will be encouraged to take an active interest in the scientific background of the project. The work will involve the use of a range of recombinant DNA and cell culture techniques.

For appropriately qualified candidates the appointment will be on the Technician Grade 3 Scale (£5,151 — £6,035).

Applications, including a curriculum vitae and the names of two referees should be sent to Dr J W Almond, Department of Microbiology, University of Leicester, University Road, Leicester LE1 7RH. (2918)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF MEDICAL CELL BIOLOGY

TECHNICIAN GRADE 3

Required immediately to assist project to study non-muscle cell motility. Work is concerned with the molecular mechanisms of control of actin filament assembly and reorganisation in cells.

Candidates must possess ONC or appropriate equivalent in a Biological discipline plus three years relevant experience. Post suitable for candidates holding degree in Biochemistry or related discipline in which Biochemistry constituted a significant part.

Post available for three years.

Initial salary within range £5,151 — £5,439 per annum. (Under Review)

Application forms may be obtained from The Registrar, The University, P O Box 147, Liverpool, L69 3BX. Quote Ref: RV/546/N (2937)A

UNIVERSITY OF BRISTOL

'NEW BLOOD LECTURESHIP'

Applications are invited for the post of Lecturer in Physics in the general area of liquid state physics with an interest in the interplay between statistical mechanical theory and experiment. Experience in some aspect of experimental liquid state physics or computer simulation would be an advantage but is not essential. The successful candidate will, however, be expected to be associated with the work of Professor J E Enderby or Dr R E Evans. Salary will be on the Lecturers' salary scale (£7,190 — £14,125), under review.

Further particulars should be obtained from the Registrar and Secretary, University of Bristol, Senate House, Bristol BS8 1TH, to whom applications should be sent by 6th July 1984, quoting reference JC. (2922)A

UNIVERSITY OF BIRMINGHAM FIELD ARCHAEOLOGICAL UNIT

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post to work on a project on decay and detection on archaeological sites.

A strong chemistry background is necessary and the ideal candidate should have a higher degree in the area of soil chemistry with an interest in Microbiology.

Post is tenable for up to three years from 1 July 1984. Salary on the Research Fellow 1A scale £7,190 — £11,615 plus superannuation.

For further particulars phone 021-472-1301 ext 2559, quoting reference F4.

No formal application form. Three copies of application, including full curriculum vitae and naming three referees to Assistant Registrar, (Science and Engineering), P O Box 363, Birmingham B15 2TT by 6 July 1984. (2948)A

CSIRO

AUSTRALIA

SOIL BIOLOGIST/ ROOT PATHOLOGIST

$A25,342-$A31,270

DIVISION OF SOILS GLEN OSMOND SA

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The **CSIRO** Division of Soils is a member of the Institute of Biological Resources.

FIELD: Soil Biology/Root Disease

GENERAL: The Division of Soils studies the physics, chemistry and biology of soils and other porous media, together with the integrative disciplines of pedology and geomorphology. It also seeks to establish principles for the application of soil science to agriculture, forestry, hydrology, engineering and conservation. The Division has laboratories in Adelaide, Brisbane, Canberra and Townsville.

The research associated with this position is funded by the South Australian State Barley and Wheat Industry Research Committees for a period of 3 years.

Field research by the Division of Soils indicates that *Rhizoctonia solani* is a serious pathogen of cereals and grain legumes in southern Australia following a change in management systems involving reduced cultivation and trash retention. Before practical control of this disease can be achieved it will be necessary to gain a greater understanding of the ecology and epidemiology of this pathogen under different soil and field conditions. The ultimate aim of this project is to develop methods of controlling the disease in the field by a variety of techniques, eg. cultivation, chemical and biological control, weed control and the screening of resistant varieties.

DUTIES: As a member of a team, conduct research on the effects of soil, tillage and rotation on root diseases of wheat on a range of soils with different rainfalls. The current program encompasses studies in the laboratory, controlled environment cabinets, glass house and in the field.

QUALIFICATIONS: A PhD degree or equivalent qualifications majoring in biological sciences or agricultural science. Relevant postgraduate experience is essential, together with expertise in root pathology and field experimentation on soil-borne root diseases.

TENURE: A term of three years. Australian Government superannuation benefits are available.

APPLICATIONS: Quoting reference No M8040, should be directed to:

The Chief
CSIRO Division of Soils
Private Bag No 2
GLEN OSMOND SA 5064
AUSTRALIA

By July 20, 1984.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W1063)A

QUEEN MARY COLLEGE University of London

Research Opportunities in Geomaterials

POSTDOCTORAL RESEARCH ASSISTANTSHIP: This SERC-funded post is to continue and further develop a major project concerned with the durability and performance of rock rubble mound breakwaters. The research involves field, laboratory and computer studies of armouring materials. Appointment for 2 years with initial salary in range (under review) £8,376 — £9,716 pa (including London Allowance).

SERC/ CASE RESEARCH STUDENTSHIP: This PhD Studentship is to continue researches concerned with deterioration and cracking of concretes due to alkali-aggregate reactivity. The work will be carried out in association with consultant engineers Mott Hay and Anderson.

Applications from suitably qualified graduates in geology, engineering geology, geochemistry or related sciences should apply by letter, with cv and names of 2 referees, to The Senior Personnel Officer, Queen Mary College, Mile End Road, London E1 4NS, by 2 July. (2978)A

The European Molecular Biology Laboratory,
a research institute situated in Heidelberg, West Germany,
invites applicants for the following vacancy

TECHNICAL ASSISTANT

The successful candidate will join a group working in the area of nucleic acid and peptide synthesis and in the development of instrumentation in these areas.

Applicants should possess a degree or equivalent qualification in chemistry and should have a strong background in organic chemistry. Technicians with an interest in biochemistry, molecular biology or physical organic chemistry are especially invited to apply. The working language will be English or German.

An above average salary will be offered to the successful candidate. Certain allowances are payable in addition, depending on personal circumstances. An initial contract of 3 year's duration which can be renewed, will be offered.

Please write briefly for an application form, quoting ref. N° 84/29 to : EMBL, Personnel Section, Postfach 10.2209, D-6900 Heidelberg (W1066)A

MRC BRAIN METABOLISM UNIT
Edinburgh
Organic Chemist

The MRC Brain Metabolism Unit invites applications from suitably qualified chemists for the post of Organic Chemist with special expertise in the synthesis of neuropeptides and appropriate analogues. Candidates of immediate post-doctoral or equivalent status will be preferred. A period (minimum 3 months) of training in the peptide chemistry in the laboratory of Dr R.C. Sheppard (MRC Laboratory of Molecular Biology) may be offered to a suitable candidate who, although highly competent in organic chemistry, has no special expertise in peptide chemistry.

The person appointed will work in close association with the Molecular Biologists in the Unit who are interested in the mechanisms of synthesis, release and mechanism of action of neuropeptides in mammalian brain. The appointment will be short-term (3 years). Remuneration will be at an appropriate point on the scales for university non-clinical academic staff.

Further details can be obtained from the Director, Dr G. Fink, MRC Brain Metabolism Unit, Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ. Four copies of applications and the names and addresses of three referees to be sent to Mrs A. Milligan, Administrative Officer, MRC Brain Metabolism Unit, Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ by Friday, July 27. (2917)A

PLANT BREEDING INSTITUTE
Maris Lane, Trumpington, Cambridge CB2 2LQ
A Post Doctoral Fellow

Sponsored by Ciba Geigy Corporation for a period three years, is required to initiate a research programme aimed at isolating transposable DNA elements from potato, in collaboration with Dr M D Bevan and Mr R E Gunn of the Institute. Emphasis will be placed on analysing changes in gene structure caused by somaclonal variation; practical experience in recombinant DNA techniques is essential and experience or an interest in mobile DNA elements would be advantageous.

Candidates should have a first or upper second class honours degree in an appropriate biological subject with at least 2 years experience in biochemistry/molecular biology or related research, and a PhD.

Appointment will be as Higher Scientific Officer (£7,149-£9,561 pa). Pay award pending. Non-contributory pension scheme.

Applications with curriculum vitae and names and addresses of three referees should be sent to the Establishment Officer, quoting reference CYT/ 182, by 19 July 1984.

The Institute is an Equal Opportunities Employer. (2959)A

THE ANIMAL VIRUS RESEARCH INSTITUTE
HEAD OF IMMUNOLOGY AND PATHOLOGY

Applications are invited for a new post of Head of the Division of Immunology and Pathology, the appointment to commence 1 October, 1984.

Candidates should have a high academic research record in molecular or cellular immunology, with experience, or an interest in, the immunology of virus infection in farm animals, and proven skills of leadership.

The Division includes Departments of immunology, experimental pathology and vaccine research and the successful candidate would also become head of the Immunology Department.

Salary in scale LSTG 16,343-LSTG 20,794 (Grade 6 previously known as Senior Principal Scientific Officer). Sick leave and non-contributory superannuation schemes. 25 days annual holiday. House may be available for suitable married applicant.

Further information, and application forms from the Secretary, Animal Virus Research Institute, Pirbright, Woking, Surrey GU24 0NF. Tel: 0483-232441. Closing date for applications 10th July, 1984. (2967)A

AGRICULTURAL AND FOOD RESEARCH COUNCIL
Institute of Animal Physiology, Babraham, Cambridge CB2 4AT
TEMPORARY MOLECULAR BIOLOGIST

Applications are invited from molecular biologists to work on the regulation of the synthesis of polypeptide factors and hormones involved in the control of animal growth and reproduction. Candidates should have postdoctoral experience of mRNA isolation and in vitro translation, cDNA synthesis and gene cloning in eukaryotic systems. Details may be obtained from K D Brown or A P F Flint (Tel: Cambridge (0223) 832312). The appointment will be at Higher Scientific Officer (£7,149 — £9,561) or Senior Scientific Officer (£8,970 — £11,476) level depending on qualifications and experience and will be for 2 years. In accordance with the provisions of the Employment Protection (Consolidation) Act 1978, staff on short-term appointments are required to waive any statutory rights to redundancy payments or to make claims of unfair dismissal, in respect of termination on the due date. Applications should be sent to the Institute Secretary, AFRC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, before 9 July 1984.

The AFRC is an Equal Opportunities Employer. (2927)A

UNIVERSITY OF LEICESTER
DEPARTMENT OF BIOCHEMISTRY
POST-DOCTORAL RESEARCH ASSOCIATE

Applications are invited for a post-doctoral position to work on the mechanisms of transformation of animal cells in culture by Rous sarcoma virus (RSV). The work will initially be directed towards clarification of the relationship between tyrosine-specific phosphorylation of the cytoskeletal protein vinculin by $pp60^{v}$-src (the transforming gene product of RSV), and other aspects of the transformed phenotype. The work may also involve analysis of the structure of vinculin and the vinculin gene.

Applicants should have a PhD in Biochemistry, and experience in some area of Tumour Biology is desirable.

Salary within the range £7,190 to £8,975.

The position is available immediately, although the starting date is negotiable, and will be for 2 years.

Applications (together with the names of two referees) should be sent to Dr D R Critchley, Department of Biochemistry, University of Leicester, University Road, Leicester LE1 7RN. (2919)A

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF GEOLOGY
DEMONSTRATOR/ SENIOR DEMONSTRATOR

Applications are invited for a Demonstrator/Senior Demonstrator in sedimentary geology in the Department of Geology. Duties include laboratory demonstrating in a wide range of undergraduate courses in sedimentology and palaeontology, field work instruction and tutorials in general geology. The successful candidate may also be required to lecture in Honours classes on specialist topics related to his/her research interests. The demonstrator will also be expected to contribute to the research activities of the department by developing his/her research interests and by interacting with staff members and post-graduate students.

Candidates should have completed, or virtually completed, their Ph.D. and be prepared to commence the position on October 1st. The appointment is for a fixed term of up to three years.

Initial salary within the range £6,310 — £8,530 per annum.

Applications (three copies), together with the names of three referees, should be received not later than 6 July, 1984 by The Registrar, The University, P O Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/545/N (2905)A

THE ANIMAL VIRUS RESEARCH INSTITUTE

HEAD OF MOLECULAR BIOLOGY

Applications are invited for a new post of Head of the Division of Molecular Biology, the appointment to commence 1 October 1984. The successful candidate would also become head of the Department of Biochemistry, and would lead a vigorous team investigating the biochemistry and genetics of several important exotic animal diseases including foot-and-mouth disease, bluetongue, and African swine fever viruses.

Candidates should have a high academic research record in the molecular biology of viruses and proven skills of leadership.

Salary in scale of lstg 16,343-lstg 20,794 (grade 6 previously known as Senior Principal Scientific Officer). Sick leave and non-contributory superannuation schemes. 25 day annual holiday. House may be available for suitable married applicant.

Further information and application forms from the Secretary, Animal Virus Research Institute, Pirbright, Woking, Surrey GU24 0NF. Tel: 0483 232441. Closing date for applications 10th July 1984. (2966)A

UNIVERSITY OF QUEENSLAND
Brisbane, Australia

PROFESSOR OF ORGANIC CHEMISTRY

Applications are invited for the Chair of Organic Chemistry. Applicants should be able to present evidence of substantial research achievement in the field of organic chemistry. The successful applicant will be expected to demonstrate and encourage excellence in research, and will be given responsibilities by the Head of the Chemistry Department for undergraduate and postgraduate teaching in the Organic Section of the Department.

Salary per annum: $A51,006. Closing date: 31 July 1984.

Please supply names of three referees.

LECTURER/ SENIOR LECTURER IN ZOOLOGY (Tenurable)

Higher degree in biology and demonstrated expertise and teaching experience in biostatistics required. Teaching and research experience in ecology or animal behaviour desirable.

Salaries per annum: Senior Lecturer $A32,677 — $A38,085, Lecturer $A24,353 — $A31,994. Closing date: 31 August 1984.

Additional information and application forms are available from the Staff Officer, University of Queensland, St Lucia, Qld 4067, Australia, or from the Secretary General, Assocation of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (W1071)A

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF BIOCHEMISTRY

RESEARCH ASSISTANT

Applications are invited for a Research Assistantship to work with Dr N J Russell on the mechanism of membrane changes in moderately halophilic bacteria during adaptation to altered salinity. The work will involve membrane and lipid isolation/characterisation, radiochemical enzyme assays and TLC/GLC analysis. Candidates should have graduated in biochemistry, microbiology or a closely related subject.

The appointment, which is for three years, is funded by a grant from the Science and Engineering Research Council, and there will be some collaboration with the University of Ottawa. Salary RA range 1B £6,310 — £7,190 per annum. Duties to commence 1st September, 1984.

Applications (2 copies), including a curriculum vitae, together with the names and addresses of two referees, should be sent to the Establishment Officer, University College, P O Box 78, Cardiff CF1 1XL. Closing date 16th July, 1984. Ref: 2794. (2921)A

THAMES POLYTECHNIC
SCHOOL OF BIOLOGICAL SCIENCES AND ENVIRONMENTAL HEALTH

LECTURESHIPS IN BIOTECHNOLOGY

SL IN MICROBIAL GENETICS

LII IN BIOLOGICAL CHEMISTRY

Applications are invited for the above posts from appropriately qualified graduates. Experience in one or more of the following is essential: molecular/microbial genetics; microbial physiology/biochemistry; fermentation technology; natural product chemistry, enzyme/cell technology.

Salary scale: £8,202 — £12,555 inclusive (under review).

SERC RESEARCH STUDENTSHIP

Applications are invited for a SERC research studentship from 1 October 1984 on a project from: biochemistry of bacterial lipopolysaccharides; molecular biology of wood-rotting fungi/lignin degradation; insect neurobiology. Applicants should have, or expect to obtain, a good honours degree in biology or related discipline. Please indicate choice of project.

Further particulars and application form from the Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6PF, to be returned by 3 July 1984.

Thames Polytechnic is an equal opportunities employer. (2915)A

CSIRO

AUSTRALIA
RESEARCH SCIENTIST/ SENIOR RESEARCH SCIENTIST
$A25,342 — $A37,274
DIVISION OF PLANT INDUSTRY
CANBERRA ACT

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Plant Industry is a member of the Institute of Biological Resources.
FIELD: Biochemistry
GENERAL: The Division conducts research fundamental to plant biology and applies its results to agricultural problems. Current research in its Biochemistry Section includes studies on chloroplast membranes, protein and nucleic acid metabolism in chloroplasts, photosynthetic carbon metabolism, the biochemistry of seed proteins, and haemoproteins involved in nitrogen fixation.
DUTIES: To conduct a program of research directed towards developing herbicides selectively effective against C_4 plants based on knowledge of biochemical processes specific to C_4 photosynthesis. The successful applicant will be expected to conduct research to expand the range of biochemical targets currently being examined, to supervise the day to day conduct of the biological screening procedures, and to co-operate with organic chemists in decisions about the direction of the synthetic program.
QUALIFICATIONS: A PhD degree or equivalent qualifications with research experience in the areas of enzymology, enzyme kinetics and structure-biological function relationships. A strong background in organic chemistry is desirable.
TENURE: Indefinite with Australian Government superannuation benefits available.
APPLICATIONS: Stating full personal and professional details with the names of at least two referees and quoting reference No A4726, should be directed to:

The Chief
CSIRO Division of Plant Industry
GPO Box 1600
CANBERRA ACT 2601
AUSTRALIA

By July 20, 1984.
CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W1064)A

PLANT MOLECULAR GENETICIST

Applications are invited for a faculty position at the Assistant or Associate Professor level in the Department of Vegetable Crops, University of California, Davis. We are seeking a Molecular Geneticist to address important problems in higher plant biology. Potential research areas might include gene expression, genome organization or gene transfer. The appointee will exploit the extensive tomato genetic resources available in the department and will be expected to contribute to the campus-wide program in molecular biology. This position is an 11-month, tenure track appointment with commitments of 15% and 85% research. Qualifications include a Ph.D. in genetics, molecular biology, or other closely allied discipline with training in molecular genetic techniques. Teaching and advising responsibilities will be within the appointee's area of specialization. Training of graduate students will be a significant responsibility.

Applicants should submit a curriculum vitae with a bibliography, reprints of pertinent publications, a statement of research and teaching interests, transcripts, and the names and addresses of at least three referees to: Dr. D. J. Nevins, Chair, Department of Vegetable Crops, University of California, Davis, California, 95616. Application deadline is September 30, 1984. Position available, December 1, 1984.

The University of California is an Affirmative Action/Equal Opportunity Employer. (NW614)A

CELLULAR BIOCHEMISTS

IMMUNEX, a Seattle based biotechnology company, is presently expanding its Cellular Biochemistry Department. The interests of this group center around the biochemical characterization of lymphokines, the development of rapid, sensitive and quantitative biological or biochemical assays for lymphokine detection, and assessment of the cellular mechanisms through which lymphokines act to stimulate or suppress cellular responses.

We are seeking candidates with a Ph.D. in Biochemistry or Cell Biology who have an interest in immune regulation and whose research training lends itself to the above goals. Training in immunology is not a prerequisite, but candidates must possess generic biochemical skills of a high order.

Immunex's multidisciplinary approach, coupled with a collegial scientific atmosphere, provides an excellent research environment. Our salaries are competitive and the benefits package includes equity participation opportunities. Please send resumes to:

Personnel Director
Immunex Corporation
51 University St.
Seattle, WA 98101 (NW619)A

immunex

UNIVERSITY OF CAMBRIDGE

PLANT BREEDING

Applications are invited for a University Demonstratorship in the Department of Applied Biology, to teach an area of Plant Breeding at undergraduate and graduate level, and engage in research, in a field other than molecular genetics. Candidates should have appropriate Honours degree and post-graduate research experience.

Further particulars may be obtained from the Administration Secretary, Department of Applied Biology, Pembroke St., Cambridge CB2 3DX (2955)A

WASHINGTON STATE UNIVERSITY

RESEARCH COORDINATOR

PhD in biochemistry or related areas to provide day-to-day guidance to a research group composed of postdocs, graduate students and technicians working on enzymology and molecular biology related to lipids and other natural products under the direction of P E Kolattukudy.

Send, by August 15, 1984, a complete CV and references to Director, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340. WSU is an Affirmative Action/Equal Opportunity employer. (NW618)A

PLANT BREEDING INSTITUTE
Maris Lane, Trumpington, Cambridge CB2 2LQ

Plant Molecular Biologist

A post-doctoral position, sponsored by Shell Research Limited for three years, is required to investigate the control of gene expression. The work will involve the dissection of functional regions of plant genes under hormonal control and their reintroduction into chromosomal DNA in modified form.

Candidates should have a first or upper second class honours degree in an appropriate biological science with at least 2 years experience in biochemistry/molecular bilogy or related research, and a PhD.

The appointment will be as Higher Scientific Officer (£7,149 – £9,561 pa). Pay award pending. Non-contributory pension scheme.

Applications with curriculum vitae and names and addresses of three referees should be sent to the Establishment Officer quoting reference CYT/ 178 by 19th July, 1984.

The Institute is an Equal Opportunities Employer. (2883)A

JAMES COOK UNIVERSITY OF NORTH QUEENSLAND

TEMPORARY LECTURER IN BIOCHEMISTRY

Applications are invited from candidates from any area of Biochemistry for appointment as Temporary Lecturer in the Department of Chemistry and Biochemistry. The position is available from January 1985 to 31 December 1986.

Applicants must have a higher degree in Biochemistry. The appointee will be engaged in teaching, research and associated duties.

The salary range for the position is $A24,353 – $A31,994 per annum and commencing salary will be determined in accordance with qualifications and experience.

Further information and application forms are available from The Registrar, James Cook University, Townsville, Qld, 4811, Australia, with whom applications close on July 20, 1984. In reply please quote Reference No. 84085. (W1062)

POSTDOCTORAL POSITIONS

YEAST AND BACILLUS

Two positions available immediately. One for studies of the expression and replication of the virus-like dsRNA Killer plasmids of S. cerevisiae. Major focus on modifications of killer cDNA's to test effects on expression and secretion of proteins. The other for studies of the cloning and expression of spo genes in B. subtilis expressed uniquely in one of the two cell compartments present late in sporulation. DNA cloning experience desirable. Salary commensurate with experience.

Send C.V. and names of 3 references to: Dr Donald J Tipper, Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01605, USA. (NW610)A

LOTHIAN HEALTH BOARD

TEMPORARY PART-TIME BASIC GRADE CYTOGENETICIST

(24 hours per week) based at the Cytogenetics Laboratory, Department of Pathology, Royal Hospital for Sick Children, Edinburgh required immediately until 20 January, 1985 during the absence of a member of staff on maternity leave.

Experince in human genetics is essential.

Further information from Miss P M Ellis, Cytogenetics Laboratory, Royal Hosptial for Sick Children, Edinburgh, telephone 031-667-1991 extension 272.

Typewritten applications, giving particulars of age, qualifications and previous experience, together with the names and addresses of two referees should reach the Area Personnel Officer, 11 Drumsheugh Gardens, Edinburgh, EG3 7QQ by 30 June, 1984. Quote M/83. (2973)A

MICHIGAN STATE UNIVERSITY

POSTDOCTORAL RESEARCH ASSOCIATE

(Biochemical Toxicology)

One Research Associate position is available immediately on an NIH sponsored three year project to study effects of xenobiotics on plasma membrane receptors, cellular calcium regulation, sodium and chloride channels, protein kinases, and ATPases.

Send Curriculum Vitae and names of three references to: Dr Fumio Matsumura, Pesticide Research Center, Michigan State University, East Lansing, MI 48824.

Michigan State University is an equal opportunity/affirmative action employer. (NW617)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
UNIVERSITY OF LONDON

DEPARTMENT OF MEDICINE

POSTDOCTORAL RESEARCH ASSISTANT

Required for up to 3 years to work with Dr Gregory Gregoriadis on a project supported by the MRC on the use of liposomes as immunological adjuvants in vaccines. Strong background in immunology an advantage/experience of liposome technology is not required.

Salary on Research Range 1A.

Applications by full curriculum vitae, quoting the names and addresses of two referees should be submitted to the School Office, RFHSM, Rowland Hill Street, London NW3 2PF. Further details available on request (telephone 01-794-0500 Extn. 4262). Closing date 13 July 1984. Please quote reference RA/GG/2. (2902)A

UNIVERSITY OF BIRMINGHAM FACULTY OF ARTS

CHAIR AND HEADSHIP OF THE DEPARTMENT OF PHYSICAL EDUCATION AND SPORTS SCIENCE

Applications are invited for the Chair and Headship of the Department of Physical Education and Sports Science which is now vacant. Applicants should have active academic interests in at least one of the Department's areas of study and research, and be willing to strengthen or establish links with other departments.

Salary in the professional range, plus superannuation.

Further particulars available from the Vice-Chancellor, University of Birmingham, P O Box 363, Birmingham B15 2TT, to whom applications (15 copies: 1 from overseas applicants) should be sent by 1st September 1984. (2928)A

UNIVERSITY OF BRISTOL

DEPARTMENT OF MATHEMATICS

Applications are invited for a

LECTURESHIP IN NUMERICAL ANALYSIS

Applicants should have a good research record and be likely to interact with present members of the Department. The salary scale for lecturers is £7,190 — £14,125 (under review) and initial salary will be determined according to age, qualifications and experience. The post is tenable from 1st October 1984 or a mutually agreeable date.

Further particulars should be obtained from the Registrar and Secretary, University of Bristol, Senate House, Bristol BS8 1TH, by whom applications should be received before 25th August 1984, quoting reference JC. (2923)A

UNIVERSITY OF NOTTINGHAM

CELL BIOLOGIST

Applications are invited for the position of Experimental Officer to join the research group developing monoclonal antibodies. The person appointed will be expect to supervise technical staff engaged in hybridoma research and should have relevant experience in cell biology and preferably immunology.

Salary will be at an appropriate point on the scale £6,310 to £9,875 per annum.

Applications including full curriculum vitae and the names and addresses of two referees should be sent to Professor R W Baldwin, Cancer Research Campaign Laboratories, University of Nottingham, Nottingham NG7 2RD. (2961)A

UNIVERSITY OF WARWICK

DEPARTMENT OF BIOLOGICAL SCIENCES

POSTDOCTORAL RESEARCH FELLOW OR RESEARCH ASSISTANT IN MOLECULAR VIROLOGY

In the Department of Biological Sciences to work on a project funded by the Medical Research Council. The work will be concerned with the application of recombinant DNA techniques to the study of Mammalian reoviruses. Salary will be on the Research 1A Scale: £7,190 — £11,615 pa or the Research 1B Scale: £6,310 to £8,530 pa. The position is available for three years from 1st August 1984.

Further information can be obtained by telephoning Drs M A McCrae or A J Easton on 0203 24011, exts 6024/6079.

Applicants should send a cv with the names and addresses of two referees to the Executive Officer, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, quoting reference MAM/AJE/NAR. Closing date 6th July 1984. (2914)A

HOWARD HUGHES MEDICAL INSTITUTE

RESEARCH ASSOCIATE/ POSTDOCTORAL FELLOW

MD or PhD sought for 8/1/84 to participate in studies on the role of insulin receptor tyrosine kinase and regulatory protein phosphorylation in insulin action. Experience preferred in the area of reconstruction of membrane proteins; cell fusion; protein chemistry or immunologic techniques.

CV and 3 references required. Salary $17,000 — $20,000/year, depending on experience.

Contact: J Avruch, Howard Hughes Medical Institute Laboratory, Diabetes Unit, Massachusetts General Hospital, Boston, MA 02114; or call: (617) 726-2874. (NW616)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(University of London)
Keppel Street WC1E 7HT

A POSTOCTORAL RESEARCH FELLOW

is required in the Immunology Unit of the Department of Medical Microbiology for a temporary post, initially for two years, to investigate the role of rheumatoid factors in chronic immune complex disease in mice, and supported by a grant from the Nuffield Foundation. Immunological experience essential. Gross salary, depending on qualifications etc, up to £8,376 (under review). Applications, consisting of full curriculum vitae and naming two professional referees, please, to Dr M E Devey at the School. (2964)A

THE HATFIELD POLYTECHNIC

SCHOOL OF NATURAL SCIENCES

LECTURER II/ SENIOR LECTURER IN PHARMACOLOGY

Applications are invited for the post of lecturer/senior lecturer with effect from 1 September 1984. Applicants should possess a higher degree in pharmacology and preference will be given to candidates with post-doctoral research experience. The successful applicant will be expected to contribute to pharmacology teaching at undergraduate and postgraduate level and will be encouraged to conduct research in a specialist aspect of pharmacology.

Salary: Lecturer II: £7,473 — £11,826. Senior Lecturer: £10,941 — £13,701.

Further particulars and application forms may be obtained from Mr R Anderson, Staffing Officer, The Hatfield Polytechnic, P O Box 109, College Lane, Hatfield, Herts. AL10 9AB. Informal enquiries may be directed to Dr B Hunt, Hatfield 68100, Ext 485. Closing date 17 July 1984. Please quote reference 789. (2932)A

Search And Selection Limited

Management Consultants

HEAD

Pharmacology and Biochemistry

A new Department combining Pharmacology and Biochemistry in associated areas of interest has been created by one of the most successful international research based pharmaceuticals companies — a mature scientist of standing is required as Head of Department; principally to manage the science — especially to inspire, motivate and coordinate the team which is some 40 strong (about equal numbers in each discipline) but also of course with responsibility for the administration intrinsic in running a Department of this size.

Considerable experience in managing other scientists, preferably in the pharmaceuticals industry, is essential and so the appointed candidate is unlikely to be younger than 35, with an established record of successful research; qualified in either Pharmacology or Biochemistry with considerable knowledge of both disciplines, holding a good first degree, and a relevant Ph.D as a prerequisite.

Location is in the south-east of England within easy reach of London. Salary guideline in the middle to later twenties with attractive 'extras' including a car.

Interested scientists are asked to send a full curriculum vitae and publications list to:—

Martin Duchesne
Search And Selection Limited,
Suite 3 Council Hall, Penn Road,
Beaconsfield, Buckinghamshire HP9 2PP.

(2933)A

SQUIBB

R&D DIRECTOR

Japan

Squibb, a major international pharmaceutical company, currently is seeking candidates for the position of R&D Director in its Japanese affiliate with offices in Tokyo. The Director must provide strong intellectual and administrative leadership to a staff of about 50 scientists who have responsibility for the registration in Japan of new human ethical and animal health products. In meeting these responsibilities, the department monitors Japanese regulatory requirements, oversees preclinical and clinical studies conducted in Japan, and prepare the necessary registration documents. Throughout this process, close liaison is maintained with the central research laboratories in the United States.

The successful candidate will have at least 15 years experience in the health care field after receiving an MD or PhD degree (or equivalent) and must be fluent in both Japanese and English.

Interested candidates should send their current resume directly to: **Mrs. Anne V. Corey, International Personnel Director, Squibb Corporation, P.O. Box 4000, Princeton, NJ 08540.** An equal opportunity employer.

(NW577)A

In der Fakultät für Physik der
ALBERT-LUDWIGS-UNIVERSITAT FREIBURG I. BRSG.
ist ein

LEHRSTUHL FÜR EXPERIMENTALPHYSIK (C 4)

(Nachfolge Osberghaus)

wieder zu besetzen.

Gedacht ist an Bewerber aus den Forschungsbereichen Atom- und Molekülphysik und deren Grenzgebieten, wie z.B Quantenoptik, Oberflächenphysik, Anwendungen der Synchrotronstrahlung.

Zusammenarbeit auf bestehenden Arbeitsgebieten (Atom-, Molekül- und Polymerphysik) ist erwünscht.

Volle Beteiligung an der Lehre in der Fakultät und an der akademischen Selbstverwaltung wird verlangt.

Bewerbungen sind bis zum 30.09.1984 zu richten an den.

Dekan der Fakultät für Physik
Hermann-Herder-Straße 3, D 7800 Freiburg i. Brsg.

(W1073)A

McGILL UNIVERSITY

MOLECULAR GENETICS GROUP

The Department of Biochemistry, Faculty of Medicine, McGill University is seeking three or four highly qualified individuals to form a research group with interests in the applications of molecular genetics to medically relevant topics. All positions are tenure track at the level of assistant or associate professor. Candidates should have a minimum of two years postdoctoral experience and demonstrated excellence in the application of recombinant DNA technology to animal cell systems. Each new member is expected to develop a separate, vigorous and competitive research program. Positions will be tenable after April 1, 1985.

Applicants are invited to send a complete curriculum vitae, copies of recent publications and the names of three references to Dr. C.P. Stanners, Department of Biochemistry, McGill University, 3655 Drummond Street, Montreal, Quebec, Canada H3G 1Y6.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada; however others are encouraged to apply. (NW612)A

Chair in Reproduction

Department of Clinical Studies, New Bolton Center
University of Pennsylvania, School of Veterinary Medicine

Both nominations and applications are invited for a newly endowed Chair, The Marion Dill and David George Jones Chair, in Reproduction at the School of Veterinary Medicine, University of Pennsylvania. This initiative represents an intent on the part of the School to build upon its already strong programme in reproduction. The successful applicant will be expected to develop an innovative programme of research in an area which is discernably relevant to the reproductive biology of farm animals. The applicant should also have the experience and interest to develop a role in leadership of the Section of Reproductive Studies at New Bolton Center. A degree in veterinary medicine is desirable but not essential.

New Bolton Center is the rural campus of the School of Veterinary Medicine and is situated 30 miles west of Philadelphia.

Interested individuals should send curriculum vitae, references and a summary of research interests to: Dr Alan M. Kelly, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia, PA 19104. Closing date for applications is September 30, 1984.

The University of Pennsylvania is an Affirmative Action, Equal Opportunity Employer. (NW609)A

CENTRAL MANCHESTER HEALTH AUTHORITY

SENIOR SCIENTIFIC OFFICER — TISSUE TYPING LABORATORY

HLA serologist to join the North West Regional Tissue Typing Laboratory to provide services for renal transplantation. Candidates should have post-doctoral experience in pre and post transplant immunological monitoring techniques, be prepared to carry out administrative duties in support of the transplant programme. The appointee will be expected to take up duties immediately.

Appointment for this permanent position will be on the Whitley council PTA Senior scale (£9,010 — £11,649).

Applications including a full CV to Dr P A Dyer, Principal Scientific Officer, Tissue Typing Laboratory, Dept. Medical Genetics, St Mary's Hospital, Hathersage Road, Manchester M13 0JH. Closing date: 2 July 1984. (2947)A

M.R.C. Clinical Research Centre
(Northwick Park Hospital)
Watford Road,
Harrow, Middlesex HA1 3UJ

Technician

There is a vacancy for a Technician to work in our Division of Clinical Cell Biology which is concerned with biochemical studies of Gastrointestinal disorders. The work will involve establishing and developing chromoatographic and electrophoretic procedures for the separation and isolation of iron transport and storage proteins. Applicants for this position should have an HNC or science degree in an appropriate subject. Salary on appointment will depend on age and experience and will be within the range £6,114-£8,370 (inclusive of London Weighting).

Application forms and further details may be obtained by writing to Miss J. Cottam, Personnel Section, quoting reference 111/2/4044.

Closing date: 13th July, 1984. (2934)A

MRC BONE RESEARCH LABORATORY, Oxford

POST DOCTORAL RESEARCH FELLOW Grade 1A

To study experimental models of human bone formation with Dr Brian Ashton and Dr Roger Smith. Preference will be given to applicants with a background of cell biology or the synthesis of extracellular matrix.

Salary in the range £7,190 — £8,530. The appointment will be for 2 years from the earliest mutually agreeable date.

Applications should be sent to Dr B.A. Ashton, MRC Bone Research Laboratory, Nuffield Orthopaedic Centre, Oxford OX3 7LD and should include a full cv and the names of two referees. (2908)A

ORGANIC SYNTHESIS DEVELOPMENT RESEARCH LABORATORY

CHEMISTS

To develop and scale up processes for synthesis of peptides. PhD or equivalent (2-5 years' experience), interest and practice in organic synthesis essential. Experience in development and/or peptide chemistry an advantage. Syntex offers competitive salaries and a comprehensive benefits package. Please send résumé and salary requirements to Syntex Corporation, Professional Employment, Dept LK, 3401 Hillview Avenue, Palo Alto, CA 94304. We are an equal opportunity employer. (NW607)A

AGRICULTURAL AND FOOD RESEARCH COUNCIL FOOD RESEARCH INSTITUTE

NUTRITION AND FOOD QUALITY DIVISION

SCIENTIFIC OFFICER

The Food Research Institute has a vacancy for a Scientific Officer to investigate the use of immunological techniques for the measurement of vitamins in foods. The person appointed will be trained in the use of these procedures and be involved in the development of these methods, their validation against currently accepted techniques and their eventual use in nutritional research studies in connection with the nutritional value of the UK diet.

Qualifications: A degree in Biochemistry or a related subject and an interest in immunological assays and their applications.

Salary: (under review) On a scale £5,682 — £7,765. Non-contributory superannuation scheme.

The Agricultural Food Research Council is an Equal Opportunities Employer.

Further particulars and application form from The Secretary, Food Research Institute, Colney Lane, Norwich, NR4 7UA, quoting reference number 84/2. Closing date: 5 July 1984. (2912)A

UMIST

RESEARCH ASSISTANT

Graduate/Post-Doctoral Research Assistant post available for two years for work, using lymphocytes, relating to molecular biological aspects of brain ageing and dementia. Previous experience and qualifications in molecular biology preferred.

Commencing salary will be according to age, qualifications and experience in the range of £6,310 — £7,630 p.a. (under review).

Applications, quoting reference OP/100/AI, including a curriculum vitae and the names of two referees should be sent to Dr R F Itzhaki, Molecular Neurobiology Lab, Dept of Ophthalmic Optics, UMIST, P O Box 88, Manchester M60 1QD.

(2975)A

MORRIS COLLEGE, a private and predominantly-black, four year liberal arts college in Sumter, S.C. is seeking two persons to teach courses. 1) a person to teach courses in general biology, genetics, ecology and other undergraduate biology courses. A PhD in biology is required. Beginning date: January 1, 1985. 2) a person to teach courses in physics with a computer science background general physics and electronic) and physical sciences. A PhD in physics required. Beginning date: August 16, 984. Salary and rank commensurate with qualifications and experience. Submit letter of application, letters of recommendation and academic transcript to: The Personnel Officer, Morris College, Sumter, S.C. 29150. Deadline for accepting applications is uly 20, 1984. An Equal Opportunity/ Affirmative Action Employer.

(NW615)A

UNIVERSITY OF LONDON

INSTITUTE OF PSYCHIATRY

DEPARTMENT OF PSYCHOLOGY

Applications are invited for a LECTURESHIP IN THE DEPARTMENT OF PSYCHOLOGY

The successful candidate will provide a service to Hilda Lewis House, a mental handicap unit based at Bethlem Royal Hospital, that has day, residential and community components. Applicants will also be expected to participate in the teaching of the Department on the M.Phil Clinical Psychology Course and in the supervision of trainees. They must be capable of taking an initiative in developing the clinical and research services of psychologists in this unit. Applicants should possess appropriate postgraduate qualification in clinical psychology and relevant experience is desirable.

Salary in accordance with the University of London Lecturer Scale £7,190 — £14,125 plus London Weighting Allowance.

Application forms and fuller job description obtainable from Anne Stevens, Department of Psychology, De Crespigny Park, London, SE5 8AF, and should be returned by Friday, 13th July, 1984. (Tel: 01-703 5411 Ex. 321). (2913)A

JOHN INNES INSTITUTE

POST DOCTORAL POSITION FOR NUCLEIC ACID CHEMIST OR MOLECULAR BIOLOGIST

Applications are invited for the above two year project funded by the Agricultural Genetics Company Limited. The work will be concerned with the development of probes for plant virus identification.

The appointment will be at Higher Scientific Officer (Salary £7,149 to £9,561 p.a.). Qualifications are a first or upper second class honours degree in chemistry, molecular biology or related subject with at least two years relevant experience.

Applications with curriculum vitae and names and addresses of three referees should be sent to The Secretary, John Innes Institute, Colney Lane, Norwich NR4 7UH by Friday 13 July 1984 quoting reference AGC/1. (2971)A

UNIVERSITY OF BRISTOL

CHAIR IN HISTOPATHOLOGY

The University invites applications for a Chair in Histopathology.

Medically qualified candidates are invited to submit applications by 14th September 1984.

Further particulars of the appointment may be obtained from the Registrar and Secretary, University Senate House, Bristol BS8 1TH.

(2957)A

UNIVERSITY OF EDINBURGH

FACULTY OF VETERINARY MEDICINE

DEPARTMENT OF VETERINARY PATHOLOGY

Applications are invited from veterinary graduates for the post of lecturer in Pathology.

The Department has an integrated approach to research and teaching in all aspects of Pathology (immunology, microbiology, morphological pathology) and has considerable expertise and facilities for work in these areas. The person appointed will be expected to contribute to teaching in pathology, to share the service commitments in diagnostic pathology (including post mortem work) and to undertake further research training in pathology leading to a higher degree (PhD or MRC Path if this has not already been obtained).

The salary scale is £7,190 — £14,125 but initial appointment will not exceed £8,975 with first increment on October 1st 1984.

Six copies of application with the names of 3 referees should be submitted to the Secretary to the University, Old College, South Bridge, Edinburgh from whom further particulars can be obtained. Closing date for applications 13th July, 1984. Please quote Reference No.1140.

(2909)A

ICI

CORPORATE BIOSCIENCE GROUP

PROTEIN BIOCHEMIST/ ENZYMOLOGIST

Applications are invited for a protein biochemist to work in the plant science section of the Corporate Bioscience Group. The successful applicant will join a team investigating aspects of cereal grain growth and will be responsible for initiating a programme to study the developmental control of enzymes of starch deposition in endosperm tissue. He or she will probably have post doctoral experience in plant protein isolation and characterisation and will have demonstrated ability to conduct independent research. His/her expertise will complement existing strengths in plant cell biology, biochemistry and recombinant DNA technology, and an ability to work in a multi-disciplinary environment will be essential.

Applications including a full cv should be directed to Miss E H Lauermann, Personnel Officer, Imperial Chemical Industries PLC PO Box No 11 The Heath, Runcorn, Cheshire. Closing date for applications is July 5th, 1984.

(2907)A

•THE BULLETIN BOARD•

Third International Nature Conference

September 10-12, 1984

Boston Park Plaza Hotel Boston, MA, U.S.A.

THE MOLECULAR BIOLOGY OF CANCER

POSTER SESSIONS

Scientists who register for the conference and wish to display posters should contact:

Posters Editor
NATURE Magazine
15 East 26 St., New York, NY 10010

Please do not telephone. Write, including a brief synopsis of poster subject and its scientific relevance. Poster space is limited, so we suggest you act promptly.

(NW468)C

TRANSGENE

TRANSGENE, an independent genetic engineering company, carries out high level research oriented towards applications of medical and industrial importance.

TRANSGENE has an opening for an experienced scientist as

HEAD OF THE FERMENTATION LABORATORY

The work will be devoted to the optimisation of fermentation process involving recombinant strains and/or cell lines.

It will require a strong interface with the research groups within the company as well as a practical understanding of aspects relevant for industrial purposes.

Therefore candidates must possess a strong background in biochemical engineering and a good expertise in fermentation and recovery development covering a range of hosts.

Salaries will be competitive and commensurate with qualification.

Knowledge of French is not necessary.

STRASBOURG is a pleasant historic town, located in the heart of Europe, very close to Germany and Switzerland.

The laboratory is located next to the University campus and enjoys close contacts with well known groups in fundamental research.

For confidential consideration, send a cv, a list of publications and indication of salary requirements to:

TRANSGÈNE S.A.
Attn J.-P. LECOCQ
11, rue de Molsheim
67000 STRASBOURG
FRANCE
Tél: (88) 22 24 90

(W1070)A

MEDICAL RESEARCH COUNCIL

Applications are invited for a short-term non-clinical scientific post in the Clinical and Population Cytogenetics Unit, tenable for 3 years. The appointee will work in a small group studying chromosome and nuclear organization, with special emphasis on relating cytological structure to molecular function. Current areas of interest include the nonhistone protein core evident in dehistonized metaphase chromosomes ('the scaffold') specific DNA protein and protein-protein interactions involved in chromosome structure and chromatin condensation, the possible role of repeated DNA sequences in chromosome organization, and the raising of monoclonal antibodies as probes of nuclear structure.

The Unit is well equipped for most aspects of cell and molecular biology including scanning and transmission electron microscopy, flow cytofluorimetry recombinant DNA technology, immunology, and computing facilities.

The successful candidate will have experience in a relevant field, and will be expected to develop his or her own independent research project within the general areas outlined above, while making use of the wide range of experimental techniques available in the Unit.

Remuneration will be at an appropriate point on the scales for University non-clinical academic staff. Applications with the names and addresses of two referees should be lodged with The Administrator, MRC Clinical and Population Cytogentics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, by Friday 20th July, 1984. Please quote the reference SCS/ 6/ 84.

(2916)A

THE MAX-PLANCK-INSTITUT FÜR KERNPHYSIK HEIDELBERG

invites applications from

ASTROPHYSICISTS or PLANETARY SCIENTISTS

under 30 years of age for a

POSTDOCTORAL RESEARCH POSITION

in theoretical cosmophysic. The current main areas of interest are interstellar dynamics, in particular the influence of cosmic rays, and the formation of stars and planetary systems. The three-year position is free from Sept. '84. The salary is based on the German civil service scale (approx. DM 40.000.-- annually).

Further details may be obtained from Prof H.J. Völk, MPI für Kernphysik, Tel. (6221) 516355. Please send applications, including a CV and the names of two academic referees, to the PERSONNEL OFFICE at MAX-PLANCK-INSTITUT für KERNPHYSIK, D 6900 Heidelberg 1, Postfach 103 980 before July 15th.

(W1077)A

PROFESSOR

The Center for Research in Veterinary Medicine is seeking a person whose primary research responsibility will be to study the agent-host interaction at the molecular and/or cellular level. The successful candidate will also participate in ongoing research projects and contribute to the training of graduate students.
A PhD degree from a recognized university in an area compatible with the responsibilities outlined above; actively involved in research and at least 3 years of post-doctoral experience in cellular or molecular microbiology. The candidate is expected to possess or to be willing to acquire a working knowledge of French. Preference will be given to Canadian citizens and permanent residents of Canada.
Commensurate with experience and with salary scale prevailing at the Institut Armand-Frappier (University of Quebec).
Please send curriculum vitae, list of publications and 3 letters of recommendation to the address below before August 31st, 1984 to: Institut Armand-Frappier, Service des ressources humaines, 531, boulevard des Prairies, Laval-des-Rapides, Laval, Québec, Canada H7N 4Z8. (NW620)A

SOUTH MANCHESTER HEALTH AUTHORITY
CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE
PATERSON LABORATORIES
DEPARTMENT OF IMMUNOLOGY

A position is available forthwith for a

POST-DOCTORAL ASSISTANT

to study the properties of cloned naturally cytotoxic human peripheral blood lymphocytes. The limited tenure of the appointment (until September 1985) is such that applicants should already have acquired considerable expertise in techniques for the purification, fractionation (including FACS IV experience) and tissue culture of human peripheral blood lymphocytes, and familiarity with the extensive literature on natural killer cells.

Salary will be in the range of £8,080 — £10,710, depending on age and experience.

Interested candidates should send a curriculum vitae immediately to Dr Michael Moore (061-445 8123 ext 416) from whom further particulars may be obtained. (2977)A

UNIVERSITY OF ST ANDREWS
DEPARTMENT OF PHYSIOLOGY & PHARMACOLOGY
TEMPORARY LECTURER

Applications are invited for a Temporary Lectureship (three years). In addition to teaching duties, the successful candidate will be expected to carry out research within the field of "muscle energetics, metabolism and locomotion of Antarctic animals'. This work is supported by an award from the NERC Antartic Research Special Topics Programme to Dr I A Johnston.

Some relevant postdoctoral or equivalent research experience in muscle research is essential. Excellent research facilities and support are available for studies of muscle physiology and biochemistry.

Salary at appropriate point within range £7,190 to £8,530 per annum (under reveiw), plus USS.

Further particulars are available from the Establishments Officer, The University, College Gate, St Andrews, Fife KY16 9AJ, to whom applications (two copies preferably in typescript) with the names of three referees, should be sent to arrive not later than 7 July 1984. (2891)A

KING'S COLLEGE LONDON
DEPARTMENT OF BIOPHYSICS
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited to work with Dr H J Gould on the isolation of human immunoglobulin gene binding proteins and the study of their properties, interactions with specific DNA sequences and function. Relevant experience (eg recombinant DNA technology, DNA cloning in eukaryotic cells, and/or transcription mapping) would be desirable.

The post, which is funded by the Leukemia Research Fund, is tenable for three years, starting as soon as possible after September, 1984. Salary in the range £7,190 — £11,615 plus £1,186 pa London allowance.

Applications, including a curriculum vitae and the names of two referees, should be sent direct to Dr Gould, Department of Biophysics, King's College London, 26-29 Drury Lane, London WC2B 5RL, before July 15. Enquiries from applicants in the US may be made to Dr Gould at the National Institutes of Health, Building 2, Room 301, Bethseda, Md 20205, before the end of June.

(2982)A

THE UNIVERSITY OF MELBOURNE

FOUR RESEARCH POSITIONS IN MOLECULAR BIOLOGY/MOLECULAR GENETICS

at the levels of

Research Fellow Grade 1 (Extendible Tenure)
Research Fellow Grade 2 (Extendible Tenure)
Senior Research Fellow (Extendible Tenure)

in the

Department of Veterinary Preclinical Sciences

Applications are invited from Molecular Biologists/Molecular Geneticists interested in the regulation of eukaryotic genes.

A sponsored Research Agreement allows the expansion of the Molecular Biology Group within the Sheep Biology Unit which comprises scientists working in immunology, embryology and physiology and will provide an excellent environment to achieve the long term goal of isolating regulatory DNA sequences for the genetic manipulation of livestock.

Positions are for 3 years with the possibility of renewal for a further period. Salaries will be in the range of $A20,991-$24,047 per annum for Research Fellow Grade 1, $A24,353-$31,994 per annum for Research Fellow Grade 2 and $32,677-$38,085 per annum for Senior Research Fellow. The level of appointment will depend on qualifications and postdoctoral research experience.

Further printed information regarding details of application procedure and conditions of appointment is available from the Appointments Officer (Academic), University of Melbourne, Parkville, Victoria 3052, Australia, or the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications, in duplicate, including names and addresses of at least three referees and quoting Position No. 260 A206, should be addressed to the Staff Officer, University of Melbourne, and close on 18 July 1984.

The University reserves the right to invite applications for the above positions and not to appoint anyone to each of the positions advertised.

(W1076)A

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
LONDON

DEPARTMENT OF PHYSIOLOGY

LECTURESHIP

Applications are invited for a lectureship in Physiology. Applicants should have an established record in research and would be expected to develop their own research interests.

The post will be held within the Joint Department of Physiology at University College London and this Medical School, where the post will be based.

Salary, according to age, qualifications and experience, within the range £8,376 — £15,312 inclusive of London Allowance.

Further information may be obtained from Professor J A Stephens, Sherrington School of Physiology, St Thomas's Hospital Medical School, London SE1 7EH tel 01-928 9292 ext 2241/7.

Applications in the form of a curriculum vitae, statement of research interests and the names and addresses of 3 referees should be sent to The Secretary, The Middlesex Hospital Medical School, London W1P 7PN by 19 July 1984. (2369)

THE UNIVERSITY OF MELBOURNE

RESEARCH FELLOW GRADE 1 (EXTENDIBLE TENURE)

in the

DEPARTMENT OF BIOCHEMISTRY

Applications are invited from people with a background in physical chemistry or physical biochemistry for appointment as Research Fellow 1. The work involves the use of photophysical techniques to characterize the dynamics of cytoskeletal networks and to study proximity relationships in biomembranes. The position is sponsored by the Australian Research Grants Scheme and is for 2 years.

Salary up to $A22,213 per annum and in the range of $A20,991 to $A24,047. For further information please contact Dr W H Sawyer, Department of Biochemistry, University of Melbourne, Parkville 3052, Victoria, Australia.

Further printed information regarding details of application procedure and conditions of appointment is available from the Appointments Officer (Academic), University of Melbourne, or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (W1067)A

INTERNATIONAL CENTER FOR MEDICAL RESEARCH FRANCEVILLE — GABON

with the objective of improving Fecondity (sterility, newborn and infant mortality, mother-child relationships, developmental problems)

RECRUITS

— a PhD, MD, or equivalent, specialist in cellular immunology, with at least 3 years post-doctoral experience in basic research.

— a Technician highly qualified in immunology, specially cellular, to act as assistant to the former. At least 5 years experience in basic research.

SUBJECT: immunology of *Loa loa* filariasis, parasitic disease endemic in West Africa, involving work on cellular mechanisms of the immune response, tests of cellular function, cytotoxicity, tissue culture (including hybridomes), T cell cloning and functional tests.

The C.I.R.M.F., created in 1979, employs 165 people of 7 nationalities. It includes departments of: immunology, biology of reproduction, microbiology, anatomo-pathology and parasitology. It has a clinical lab (17,000 samples/year) and gynecological-clinical facilities (30 beds) at the General Hospital. It possesses the most modern investigation equipment and harbours a Primatology Centre with 225 monkeys of 6 species.

CONDITIONS

— Two-year contract, renewable by mutual agreement, commencing September 1984. Attractive salary, negotiable, depending on qualifications and experience, plus expatriation allowance. Knowledge of French an advantage.

— Sixty day annual leave including family transportation, free housing in a complex with tennis and swimming-pool.

For further information or application, send C.V., list of publications, list of 3 referees, present salary to:

The Director of C.I.R.M.F.
28 boulevard de Grenelle,
75015 PARIS (France).

An interview with candidates under consideration will be held in Europe in July or August.

(W1068)A

University of California Lawrence Berkeley Laboratory Announces an opening 1 January 1985 for Associate Laboratory Director and Head of Nuclear Science Division

The Lawrence Berkeley Laboratory is a multi-purpose research laboratory funded by the Department of Energy. Its Nuclear Science Division conducts a multi-million dollar research program in low, medium and high-energy nuclear physics. The Head of the Division has primary responsibility for the leadership and management of this program under the general direction of the Deputy Director for General Sciences and the Laboratory Director. As an Associate Director of the Laboratory, the incumbent will also participate in formulating the research policy and long-term direction of the Laboratory as a whole. Candidates must have had significant experience as practicing nuclear physicists and be of the general calibre of persons holding senior tenure positions in major research institutions. The successful applicant would be expected to serve as Associate Director and Division Head for an initial term of 3 to 5 years, subject to renewal for further terms by mutual agreement.

The Laboratory is seeking a respected and energetic nuclear physicist willing to play a strong leadership role at a challenging time. Qualifications must be such that it will be natural to consider him or her for a tenured appointment in the Department of Physics on the Berkeley campus of the University of California, as well as at the Laboratory. Interested applicants should submit a curriculum vitae, together with the names of three references to: Dr J.D. Jackson, Chairman, Nuclear Science Search Committee, c/o Employment Office, LAWRENCE BERKELEY LABORATORY, 1 Cyclotron Road, Berkeley, CA94720. Refer to Job A/2947. An Equal Opportunity Employer M/F/H. Deadline for applications: August 31, 1984. (NW594)A

LAWRENCE BERKELEY LABORATORY

FERMENTATION CONSULTANT

We are a rapidly growing biotechnology firm manufacturing and selling enzymes and other process aids to the food and drink industries worldwide. Our manufacturing facilities are located in Western Europe and we are currently engaged in expanding our fermentation capacity substantially. We are seeking the services of an experienced fermentation technologist to act as consultant.

The responsibilities of this consultancy will be two-fold (1) to oversee/advise on the commissioning of our new plant and (2) to contribute technically at the pilot and production scale to the optimisation of microbial (bacterial and fungal) fermentation processes currently under development.

Application and correspondence should be submitted to the undersigned below and will be treated in strictest confidence. Keith Walesby, Bio/ Technology, 4, Little Essex Street, London, UK, WC2R 3LF.

(W10078)A

nature classified

LONDON OFFICE
Keith Walesby
4 Little Essex Street
London WC2R 3LF
Tel: 01 240 1101
Telex: 262024

NEW YORK OFFICE
Miss Gene Fein
15 East 26 Street
New York, NY 10010
Tel: (212) 689 5900

TORONTO OFFICE
Peter Drake
Associates
32 Front Street West
Suite 200, Toronto,
Ontario M5J 1C5
Tel: (416) 364 1623

UNIVERSITY OF DUBLIN
Trinity College

Department of Immunology

POST-DOCTORAL RESEARCH FELLOW

Applications are invited for the above post in the Department of Immunology at Trinity College, Dublin. The successful candidate will work on a research project concerned with the immunological aspects of respiratory disease. Experience in immunological technology is desirable, though not essential.

Candidates should submit a full curriculum vitae, together with the names of two referees, to:

Dr Conleth Feighery, Department of Immunology, Central Pathology Laboratory, St. James's Hospital, Dublin 8

to arrive not later than 29th June, 1984. (2882)A

THE UNIVERSITY OF WESTERN AUSTRALIA
PERTH
LECTURER IN ZOOLOGY (MAMMALIAN ECOLOGY) (FIXED FERM)

Applications are invited from suitably qualified persons for appointment for three years with some prospects of continuation to a Lectureship in the Department of Zoology. The Department has a tradition of marsupial studies in this appointment wishes to emphasize marsupial population biology. Experpertise in mammalian population biology is sought; previous marsupial experience is not required. The Department runs a number of field stations suitable for the study of marsupial populations. The appointee will lecture to undergraduates generally and in a specialist unit, undertake research, and will be encouraged to lead graduate students.

Current salary range: A24353-$A31994 per annum. Benefits include superannuation, fares to Perth for appointee and dependent family and removal allowance. Conditions of appointment will be specified in any offer of appointment which may be made as a result of this advertisement.

Applications in duplicate stating full personal particulars, qualifications and experience and the names and addresses of three referees, should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia 6009, by 21 July 1984. (W1059)A

Please mention
nature
when replying to these advertisements

UWIST
UNIVERSITY OF WALES

APPLIED BIOLOGY RESEARCH ASSOCIATE (fixed term — 2 years)

supported by the National Kidney Research Fund to work on the regulation of intracellular protein degradation in kidney epithelial cell culture. Experience in tissue culture is desirable.

Salary: Within Range 1A Research and Analogous Staff £7,190 — £8,080 per annum.

Requests (quoting Ref B67) for details and application form to Staffing Office, UWIST, PO Box 68, Cardiff CF1 3XA.

Closing date: 5 July, 1984. (2901)A

CONFERENCES and COURSES

UNIVERSITY OF NOTTINGHAM

MSc COURSE IN AGRONOMY
(the science and practice of crop production)

Applications are invited from Graduates in Agriculture, Horticulture, Agricultural Botany, Plant Science, Ecology, Botany or Biology or other appropriate science subjects for admission to an advanced course of study leading to the degree of MSc (by examination) in Agronomy. The course is designed to provide those having appropriate scientific training with specialist knowledge of agronomy.

The course is of eighteen months duration commencing in January and ending in July of the following year and it consists of four parts:- Principles I includes studies of crop nutrition, fertilizers, weed biology and control, crop water relations and irrigation, variety testing and plant breeders' rights.

Principles II includes computing, crop environment, climatology, biometry, principles of crop protection and crop physiology.

Production I and II provide a detailed study of the production, mechanisation and utilization of agricultural and horticultural crops and grassland, together with field experimentation. In additon each candidate will carry out a research project on an agronomic topic approved by the Head of the Department and will be required to submit a thesis.

For suitable UK applicants, scholarships may be available.

Those seeking admission for the 1985-86 session should write before 15th October 1984 requesting an application form from The Secretary (Ref PGA), School of Agriculture, University of Nottingham, Sutton Bonington, Loughborough, Leics LE12 5RD. (Tel Nottingham 506101 Ext 8156). (2944)C

AWARDS



G.B. MORGAGNI PRIZES

The Fidia Research Laboratories and the Faculty of Medicine of the University of Padua announce the establishment of the G.B. Morgagni Prizes, consisting of one Morgagni Medal and two Young Investigator Awards. These prizes will be awarded every four years on the occasion of the European Symposium on Metabolism, Padua, Italy, commencing in 1985.

Nominations are invited for the Morgagni Medal which is to be awarded to an outstanding European scientist working in the field of metabolism, including metabolic diseases such as obesity, diabetes and hyperlipidemia. The first Medal and US$ 15.000 will be presented at the Fourth European Symposium on Metabolism in Padua, Italy, in May 1985. Each nominee should be nominated by two individuals, who should enclose the nominee's curriculum vitae and send supporting statements of not more than 500 words each. The winner will present a lecture on the occasion of receiving the Medal.

Applications are also invited from European scientists below the age of 40 on January 1st, 1985 and working in the field of metabolism for two Young Investigator Awards, each valued at US$ 5.000, which will be given at the same time as the Morgagni Medal. Applicants should enclose a curriculum vitae and three reprints of the most important papers.

Nominations for the G.B. Morgagni Medal and applications for the Young Investigator Awards should be sent before November 30th, 1984 to:

The Secretary
The G.B. Morgagni Prizes Committee
Istituto di Medicina Interna
Via Giustiniani, 2
35100 - Padova (Italy)

(W1069)N

GRANTS and SCHOLARSHIPS

Requests for Applications For Research Funds

The Health Effects Institute* (HEI) *announces its intention to make available requests for applications for research funds.

The Health Effects Institute is a non-profit research organization founded in 1980. It was established co-operatively by the United States Environmental Protection Agency and the automotive industry but is governed by an independent 3-member Board of Directors: Archibald Cox, Carl M. Loeb Professor, Harvard University; William O. Baker, Chairman Emeritus, Bell Laboratories; and Donald Kennedy, President, Stanford University. The HEI funds research directed to understanding and evaluating the health effects of mobile source emissions. The research program was developed by HEI's Health Research Committee whose chairman is Walter Rosenblith, Institute Professor and former Provost of the Massachusetts Institute of Technology.

The Health Effects Institute currently has thirty studies underway at 25 universities and research centers in 14 states and in West Germany.

This set of research topics is the first set of requests to be issued under the Institute's Phase Two Research Agenda. The topics will be addressed through full research applications on the following subjects:

1. **Mechanisms of carbon monoxide toxicity**
2. **Acute effects of carbon monoxide on cardiac rhythm**
3. **Mechanisms of oxidant injury**

And preliminary applications on:

4. **New methods for assessing health risks from automotive emissions**

In addition to these topics, the HEI will also consider applications in other areas. These include full applications from new investigators and preliminary applications from established investigators on novel and important research in connection with the health effects of automotive emissions.

The Health Effects Institute will make available a packet of descriptive material about the research topics, directions for preparing applications, and material about the Institute to all interested investigators.

All requests and inquiries should be made to or through:
Dr. C. W. Powers, Executive Director, The Health Effects Institute, 215 First Street, Cambridge, Massachusetts, 02142, 617/491-2926.

(NW621)H

UNIVERSITY OF LIVERPOOL
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

WOLFSON UNIT OF MOLECULAR GENETICS

POSTGRADUATE SCHOLARSHIP

Applications are invited for a postgraduate research studentship to investigate the molecular biology of microorganisms which determine mating incompatibility in mosquito vectors of disease. Applicants should hold, or expect to gain this summer, a First or Upper Second Class Honours degree in a Biological Science. Experience in techniques of molecular biology would be useful but not essential.

The successful applicant will be expected to register for a higher degree and will join a group working on the application of recombinant DNA techniques to the study of malaria, leishmaniasis and the insect vectors of infections of man.

Applications, including the names of two referees, should be received by The Registrar, The University, P O Box 147, Liverpool L69 3BX. Quote Ref: RV/541. (2899)H

SEMINARS and SYMPOSIA

BIODETERIORATION SOCIETY SUMMER MEETING

Microbial Corrosion of Metals
University of Kent at Canterbury
July 5th and 6th 1984

For Programme details contact:
The Secretary
Biodeterioration Society
Biological Sciences
Hatfield Polytechnic
Herts AL10 9AB

(2906)M

14-26 April, 1985

The 10th Edmond de Rothschild School in Molecular Biophysics:

THE STRUCTURE OF DNA AND OF CHROMATIN, Israel.

Weizmann Institute of Science and Neve Ilan Hilltop Resort, near Jerusalem.

Mrs. Ruth Goldstein, Conference Coordinator, Aharon Katzir-Katchalsky Center, Weizmann Institute of Science, 76 100 Rehovot, Israel. (W1072)M

FELLOWSHIPS

THE UNIVERSITY OF LEICESTER

DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL FELLOWSHIP

Applications are invited for a post-doctoral research position (MRC-funded) to work on the interaction between tetanus toxin and neural tissue. The project will include studies on (1) the "receptor" for tetanus toxin (2) the biochemical and ultrastructural features of the internalization process and (3) structure function relationships within the toxin molecule itself.

Techniques involved will be in part similar to those previously developed to study the interaction of cholera toxin with target tissues (J. Biol. Chem.256 (1981) 8724), but will also involve the extensive use of monoclonal antibodies.

Applicants should have a PhD in Biochemistry or related discipline, and preference may be given to those with experience in cell culture and immunochemical techniques, and with an interest in membranes.

Salary: £7,190 to £8,080

The position is immediately available although the starting date is negotiable, and will be for a maximum of 3 years.

Applications (along with the names of two referees) should be sent to Dr D R Critchley, Department of Biochemistry, University of Leicester, University Road, Leicester LE1 7RN. (2920)E

UNIVERSITY OF OTAGO

DUNEDIN — NEW ZEALAND

POST-DOCTORAL FELLOWSHIPS

Applications are invited for Post-Doctoral Fellowships in 1985 to be funded and awarded by the University Grants Committee and tenable within the University of Otago.

Awards may be made for one year or for three years.

Applications from candidates wishing to work in any department of the University of Otago will be considered, but in making the awards the University Grants Committee will give priority to current growth research areas such biotechnology, genetic engineering, electronics, engineering, chemistry, etc.

The emolument payable will be at a rate to be determined but not exceeding the lowest step on the University scale for lecturers (currently NZ$21,660 per annum).

Applicants should have completed the requirements for the award of the degree of Doctor of Philosophy.

Application forms and further particulars are available from the Secetary-General, Association of Commonwealth Universities (Appointments), 36 Gordon Square, London WC1H 0PF. Applications close with the undersigned, PO Box 56, Dunedin, New Zealand on 23 July 1984. D W Girvan, Registrar.

(NW623)E

Southampton THE UNIVERSITY

INSTITUTE OF CRYOGENICS

RESEARCH FELLOWSHIP

Applications are invited for a 2 year research fellowship supported by SERC to study convection in cryogenic fluids using Laser Doppler Anemometry and microthermometry. Experience with data processing and lasers preferred.

Salary on range: £7,190 – £8,080 per annum (under review).

Applications giving Curriculum Vitae (in duplicate) and names of two referees should be sent to D.A.S. Copland, Southampton University, Highfield, Southampton SO9 5NH, as soon as possible quoting reference 2056. (2904)E

UNIVERSITY OF WARWICK

DEPARTMENT OF BIOLOGICAL SCIENCES

POSTDOCTORAL RESEARCH FELLOW

A Cancer Research Campaig funded fellowship is available t work with Dr A G Morris in the stud of cell- and molecular-biologica aspects of the production of gamm interferon by human and murin lymphocytes. The position would su best either an immunologist or molecular biologist with an interest i cellular immunology, but an biologist with an interest in the are would be considered. The position tenable for one year, in the first in stance, renewable for at least on further year. The salary will be on th Research IA scale: £7,190 to £11,61 pa. Applicants should send a cv, wit the names and addresses of tw referees, to the Executive Office Department of Biological Science University of Warwick, Coventr CV4 7AL, quoting reference AGM NAR/BS554. (2926)E

UNIVERSITY OF EDINBURGH

DEPARTMENT OF ZOOLOGY

POST-DOCTORAL FELLOWSHIP IN BIOCHEMISTRY

Applications are invited for a SER Fellowship to work on th biochemistry of the yeast cell cycl Research range IA starting 1 Octob 1984.

Further particulars are availab from Professor J M Mitchiso Department of Zoology, Universi of Edinburgh, Kings Buildings, We Mains Road, Edinburgh EH9 3J with whom applications (2 copie including CV and the names of tw referees should be lodged not lat than 9 July 1984.

Please quote reference No 5178. (2311)E

FELLOWSHIPS *continued*

UNIVERSITY OF WATERLOO
Department of Biology
NSERC University Research Fellows

The Department of Biology, University of Waterloo, invites applications for NSERC University Research Fellowships in the following areas of research interest: **(i)** population genetics with emphasis on the genetic structure and dynamics of real as opposed to theoretical populations; **(ii)** nucleic-acid chemistry with emphasis on either *in vitro* DNA synthesis or site-specific mutagenesis; **(iii)** protein chemistry with emphasis on immunological analyses. Applications in other areas will also be given serious consideration. Successful candidates will be expected to establish a vigorous research program and participate in teaching both undergraduate and graduate students.

A curriculum vitae including names of three referees should be sent to Dr. J.E. Thompson, Chairman, Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 3GL. Prospective candidates should examine the NSERC Awards Guide 1984-85, paragraph 186 on page 52 for details.

In accordance with Canadian Immigration requirements, this advertisement is directed to Canadian citizens and permanent residents. (NW611)E

MERTON COLLEGE, OXFORD
JUNIOR RESEARCH FELLOWSHIPS AND SENIOR SCHOLARSHIPS 1985

The college proposes, if candidates of sufficient merit present themselves, to elect to two or three Junior Research Fellowships and to two or three Senior Scholarships in 1985. These awards are open to both men and women. Candidates for Junior Research Fellowships must be under the age of 27 on 1 October 1985; candidates for Senior Scholarships must be under the age of 26, or, in the case of Oxford graduates, must not have exceeded 18 terms from the date of matriculation.

Details of the awards and of the method of appying for them may be obtained from the Warden's secretary, Merton College, Oxford, OX1 4JD. The closing date for applications is SATURDAY 20 OCTOBER 1984. (2929)E

AUSTRALIA

Queen Elizabeth II Fellowships Scheme

PHYSICAL & BIOLOGICAL SCIENCES

To commemorate the Royal Visit to Australia in 1963 the Australian Government established the Queen Elizabeth II Fellowships Scheme. Under this scheme up to ten fellowships may be awarded each year for full-time research by young scientists of exceptional promise and proven capacity for original work. These are post-doctoral awards tenable in an Australian university or approved research institution normally for two years. Tenure of a Fellowship is expected to commence within nine months of the announcement of the award.

QUALIFICATIONS Queen Elizabeth II Fellows must be either Australian or United Kingdom citizens. They should have a PhD., or equivalent qualifications, in one of the physical or biological sciences (which are deemed to include mathematics and the scientific aspects of statistics, engineering, metallurgy, agriculture and medicine). In some circumstances applications may be considered after submission of a thesis, but before the degree has been awarded.

Awards will, in general, be restricted to applicants who are not more than 30 years of age on the date when applications close.

SALARY $25,447 (Australian) per annum — increased to $27,628 per annum at age 28 years.

TRAVEL EXPENSES Necessary travel expenses are payable.

APPLICATIONS Persons interested in applying for the above fellowships should obtain application forms and a statement of the conditions of award from:
Executive Officer, Queen Elizabeth II Fellowships, Department of Science and Technology, P.O. Box 65, Belconnen, A.C.T. 2616, Australia.

The next round of awards will be announced in December 1984. Applications received after the closing date of 15 August 1984 will not be considered. (W1065)E

66181

The Queen's University of Belfast
Postdoctoral Research Fellowship in Food Bacteriology
Department of Agriculture and Food Bacteriology

This MAFF supported Fellowship under the direction of Dr. J.T. Patterson will involve work on the extension of the shelf-life and improvement in consumer appeal of processed meat products. Both microbial and biochemical assessments will be used. The appointment, which is for one year initially, subject to renewal for a further year, is available as soon as possible.
Salary in the range: £7,191-£8,532 per annum with contributory pension rights under USS.
Applications providing full details of qualifications and research experience together with the names and addresses of two referees should be sent to the Personnel Office, The Queen's University of Belfast, Northern Ireland, BT7 1NN, as soon as possible. (Please quote Ref: 84/ N.) (2956)E

THE UNIVERSITY OF AUCKLAND
New Zealand
POSTDOCTORAL FELLOWSHIPS

Applications are invited for Postdoctoral Fellowships to commence during 1985. Applications in any research field (including humanities and social sciences) will be welcomed but preference will be given to those in current growth research areas — biotechnology, genetic engineering, electronics, etc, or in developing fields of economic importance to New Zealand such as tourism.

The Fellowships are available for either one or three years and are valued at NZ$21,660 per annum. Conditions of Appointment and Application Forms are available from the Assistant Registrar (Academic Appointments), University of Auckland, Private Bag, Auckland, New Zealand, or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications should be forwarded to the University as soon as possible but not later than 28 July 1984. (W1078)E

NUFFIELD FOUNDATION
ONE YEAR SCIENCE RESEARCH FELLOWSHIPS

The Nuffield Foundation is offering to University Lecturers in Science and Applied Science Departments a number of one-year research fellowships to be awarded competitively at the end of 1984 and to be held during the academic year 1985-86. The objective is to enable younger university lecturers to be freed from teaching and administrative duties for one academic year. Preference will be given to applicants under the age of 40. Awards will cover the cost of replacement teaching (up to the fourth point on the lecturer scale) and will include an allowance for research expenses.

The closing date is 14th October 1984 and further particulars may be had from the Director, Nuffield Foundation, Nuffield Lodge, Regent's Park, London NW1 4RS.

This scheme is only open to applicants from Institutes within the United Kingdom. (2951)E

STUDENTSHIPS

SUNDERLAND POLYTECHNIC
DEPARTMENT OF BIOLOGY

SERC Research Studentship

Applications are invited for this studentship (supervised by Dr. W. Arthur and tenable for three years from 1 October 1984) on evolutionary and ecological aspects of competition between species of *Drosophila*.

Applicants should hold a first class or upper second class honours degree in a biological science.

Further particulars available from Personnel Officer, Sunderland Polytechnic, Ryhope Road, Sunderland SR2 7EE, or tel: 0783 76233 ext. 11. Closing date for receipt of applications: 5th July, 1984. (2942)F

UNIVERSITY OF SOUTHAMPTON
Clinical Pharmacology/ Medicine I

SERC (CASE) STUDENTSHIP
Culture and Biochemistry of Human Mucosal Mast Cells

Applications are invited from good honours graduates in biochemistry or related biological sciences to establish tissue culture methods for human mucosal mast cells and to explore the secretory characteristics and biochemical pharmacology of the cells. An SERC (CASE) studentship in collaboration with Roussel Labs is available for 3 years and the successful applicant will be expected to register for a higher degree.

Further information and applications, including curriculum vitae and the names of two referees to: Dr M K Church, Clinical Pharmacology, Southampton General Hospital, Southampton SO9 4XY. Telephone 0703 777222 ext. 4312. Applications close 6th July 1984. Please quote Ref: AJS/84/25/N (2945)F

UNIVERSITY COLLEGE CARDIFF
DEPARTMENT OF PHYSIOLOGY

RESEARCH STUDENTSHIPS

A three year MRC Studentship and an SERC CASE Studentship are available from 1st October 1984. The MRC studentship concerns the properties of primary afferent neurones innervating cranial blood vessels, the meninges and their possible involvement in migraine headache. The project will involve electrophysiological and histological techniques, and is under the supervision of Professor D I Wallis.

The SERC CASE studentship is concerned with the characterisation of 5-HT receptors in the CNS using electrophysiological methods in vivo, and is under the supervision of Dr M H T Roberts.

Applicants should possess, or expect to obtain a First or Upper Second Class Degree in Physiology, Pharmacology or some other appropriate subject.

Those interested should write as soon as possible to Professor D I Wallis or Dr M H T Roberts, Department of Physiology, University College, P O Box 78, Cardiff, CF1 1XL. Ref: 2798. (2940)F

WELSH NATIONAL SCHOOL OF MEDICINE
UNIVERSITY OF WALES
DEPARTMENT OF MEDICINE
SECTION OF MEDICAL GENETICS

RESEARCH STUDENTSHIP IN MOLECULAR GENETICS

Applications are invited for a 3 year position leading to a PhD degree, on the application of recombinant DNA techniques to the analysis of human inherited diseases, in particular myotonic dystrophy and neurofibromatosis.

Further details may be obtained from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN. (Tel 0222/755944 Ext 2296) quoting Ref No M6/117 to whom application in the form of a curriculum vitae with the names and addresses of two referees should be submitted. (2924)F

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF METALLURGY AND MATERIALS SCIENCE

RESEARCH STUDENTSHIPS

Applications are invited from good honours (1st or 2(i)) graduates in Metallurgy/Materials Science or related Physical Sciences for 3 year studentships tenable from October 1 1984. The studentships are sponsored by the SERC Quota and CASE schemes, and are in the following areas:

(i) solidification processes in high temperature alloys;

(ii) theoretical and experimental studies of irradiation damage effects in metals;

(iii) modelling deformation and fracture mechanisms in composite materials;

(iv) interfacial structure in semiconductor materials;

(v) structure of complex oxides for advanced batteries;

(vi) structure/property relationships in polymers;

(vii) environmental effects on the strength of graphite.

(viii) embrittlement sensitivity of 9Cr-IMO steel.

Applicants interested in any of these projects should write to The Registrar, The University, P O Box 147, Liverpool, L69 3BX. Quote Ref: RV/543. (2900)F

TRENT POLYTECHNIC
THE DEPARTMENT OF LIFE SCIENCES

invites applications for SERC studentships for work in the following fields of study.

Microbial degradation of xenobiotic chemicals, Neurotransmitter pharmacology, Calcitonin control of blood pressure, Drug transport in liver and kidney, Controlled reproduction of coarse fish, Urea cycle, lysine and ornithine metabolism, Ageing in stored potatoes, Effects of acid rain on amphibia, Physiology and biochemistry of germinating seeds.

Further details and form of application are available from the Staff Officer, Trent Polytechnic, Burton Street, Nottingham NG1 4BU. Applications to be returned as soon as possible. (2981)F

UNIVERSITY OF ASTON
DEPARTMENT OF BIOLOGICAL SCIENCES

RESEARCH STUDENTSHIP

Applications are invited for the above three-year appointment to undertake research, with Dr Hanson and Dr Bailey, into the causes and effects of diabetes and obesity using genetically obese mice.

Applicants should possess, or expect to attain, a degree of Class 1 or 2 (i) in Biochemistry/Physiology or a related discipline.

Further particulars are available from Dr P J Hanson, Department of Biological Sciences, University of Aston, Gosta Green, Birmingham B4 7ET (Tel 021-359 3611 ext 5330), to whom applicants should send a curriculum vitae, together with the names and addresses of two academic referees, by July 6th, 1984. (2974)F

RESEARCH STUDENTSHIPS

A three-year research studentship for the session 1984/85 is available to a suitably qualified candidate (B.Sc, Class 1 or 2:1). The student will be registered for a PhD and will work in the Department of Dermatology under the the supervision of Dr G C Priestley. The award comprises a maintenance grant of £2,430 per annum, plus fees and a small travel allowance.

The project will involve studies of the growth and metabolism of fibroblasts cultured from the skin of patients with scleroderma and lichen sclerosus. Both two- and three-dimensional culture systems will be employed and a range of analytical techniques are available.

Applications should be made to Dr G C Priestley, Department of Dermatology, Phase I, The Royal Infirmary, Edinburgh, EH3 9YW, or by telephone 031-229-2477 Ext 2430, not later than 28 June 1984. (2911)F

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF DENTAL SCIENCES

MRC RESEARCH STUDENTSHIP

Applications are invited from graduates who hold or expect to obtain a first or upper second class honours degree in a biological discipline including an adequate background in biochemistry. A number of research topics are available including studies of the connective tissues of the mouth in relation to periodontal disease and the structure and biosynthesis of salivary glycoproteins and their role in dental plaque formation. The latter project would give scope for a microbiologist.

The successful applicant would join an active team under the direction of Dr G Embery, to whom enquiries may be made. Applications together with the names of two referees should be sent not later than July 9, 1984, to the Registrar, The University, P O Box 147, Liverpool L69 3BX. Quote Ref: RV/548/N. (2946)F

UNIVERSITY OF OXFORD
NUFFIELD DEPARTMENT OF ORTHOPAEDIC SURGERY
MRC BONE RESEARCH LABORATORY

Applications are invited for a

MRC RESEARCH STUDENTSHIP

to join an active research team for work on the differentiation of bone forming cells in normal and pathological conditions with particular reference to the characterisation of cell-specific markers by using monoclonal antibody techniques. Applicants should have, or expect to obtain, a first or upper second class honours degree in a biological science, preferably biochemistry, and will be expected to register for a DPhil degree.

The names and addresses of two referees and a completed curriculum vitae should be sent as soon as possible to Dr J T Triffitt, MRC Bone Research Laboratory, Nuffield Orthopaedic Centre, Oxford OX3 7LD (2938)F

UNIVERSITY OF ASTON IN BIRMINGHAM
DEPARTMENT OF BIOLOGICAL SCIENCES

FACULTY STUDENTSHIP

Applications are invited for a Faculty studentship leading to the degree of PhD commencing October 1984 in the area of experimental and clinical Diabetes Mellitus under the supervision of Dr T Atkins. Candidates should have, or expect to have a first or upper second class honours degree in Biochemistry/Physiology or a related subject.

Applications which should include a curriculum vitae, telephone number and the names of two referees, should be sent to Dr T Atkins, Department of Biological Sciences, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET (2952)F

STUDENTSHIPS

BRUNEL UNIVERSITY

Cell Biology Research Group

Applications are invited for a postgraduate research studentship, funded by the Cystic Fibrosis Research Trust, for a study of evasion of macrophage functions by Pseudomonas aeruginosa. The project will be supervised jointly by Drs Susan Smith and Roger Dean. The successful candidate will be registered for a PhD degree and should hold or expect to obtain a good honour degree in an appropriate subject.

Applications, including a curriculum vitae and the names of two referees, should be sent to Dr S E Smith, School of Biological Sciences, Uxbridge, Middlesex UB8 3PH, from whom further information is also available. (2943)F

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF PHYSIOLOGY

RESEARCH STUDENTSHIPS

Projects available in muscle physiology, cortical neurophysiology and developmental neurobiology. Facilities for work in vivo and in vitro.

Postgraduate Advisor, Department of Physiology, UCL, Gower Street, London WC1E 6BT. (2950)F

PROPOSED UNIVERSITY OF ULSTER
Faculty of Science

SERC CASE STUDENTSHIP IN PHYSICS
(in collaboration with Culham Laboratory)

Applications are invited for a research Studentship to study the production of negative hydrogen ions in plasma sources. The project will involve the student in the design, operation and diagnosis of plasma generators and extraction systems for the intense neutral beam injection systems for heating nuclear fusion plasmas. The work will be carried out at both the Coleraine Campus of the University and the Culham Laboratory.

Candidates should have or expect to obtain an upper second class or first class honours degree in physics or a similar discipline.

Further details may be obtained from the Academic Registrar (Designate), The University of Ulster, PO Box 35, Coleraine, Northern Ireland BT52 1SA, to whom applications, in the form of a curriculum vitae and the names and addresses of two referees, should be sent by 11th July, 1984.

Note: a petition for a charter for the proposed University of Ulster has been submitted to the Privy Council by the New University of Ulster and the Ulster Polytechnic. The petition proposes the merger of the two institutions with effect from 1st October, 1984. (2983)F

UNIVERSITY OF CAMBRIDGE

RESEARCH STUDENTSHIP IN ORGANIC CHEMISTRY OR BIOCHEMISTRY

Applications are invited from those graduating in the above fields to work on the synthesis and testing of novel arsonic acids, in which the $-CH_2-AsO_3H_2$ group replaces the $-O-PO_3H_2$ group of natural metabolic intermediates. Such analogues, e.g. of nucleotides and glycolytic intermediates, can be 'uncoupling agents', because they can be enzymically phosphorylated to form compounds that spontaneously hydrolyse to the starting analogues.

Applicants should be eligible to hold an SERC training grant. They should send a Curriculum Vitae and ask two referees to write about them, by Monday, 9 July to Dr H B F Dixon, Department of Biochemistry, Tennis Court Road, Cambridge, CB2 1QW; further details are available from him. (2972)A

UNIVERSITY OF CAMBRIDGE
Division of Virology
DEPARTMENT OF PATHOLOGY

POST-GRADUATE STUDENTSHIP IN VIROLOGY

Applications are invited for an MRC-funded studentship starting 1 October 1984. The project is to investigate interactions between herpesvirus proteins and antiherpes drugs and will involve the cloning, in vitro manipulation and expression of herpes genes in E coli.

Applicants should have or expect to obtain a first or upper second class honours degree in a biological science and should send a curriculum vitae together with the names and addresses of two referees as soon as possible to Dr G Darby, Division of Virology, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP. (2980)F

NATIONAL INSTITUTE FOR MEDICAL RESEARCH
POSTGRADUATE RESEARCH STUDENTSHIPS

Applications are invited for two MRC-funded postgraduate research studentships tenable from October 1984, in the divisions of Parasitology and Physical Biochemistry. The projects are entitled:

1. The identification and molecular cloning of stage-specific genes of Trichinella spiralis (supervised by Dr A J G Simpson and Dr S R Smithers)

2. Energy transduction in muscle (supervised by Dr M A Ferenczi)

Candidates should have, or expect to obtain, a first or upper second class honours degree in a biological science subject.

Applications including a Curriculum Vitae and nominating two academic referees should be made to the Director of Postgraduate Studies, at the National Institute for Medical Research, before 10th July 1984.

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF BIOCHEMISTRY

SGRC STUDENTSHIP IN STEROID BIOCHEMISTRY

Applications are invited for the above studentship, tenable for three years commencing October 1984. Candidates must hold, or expect to obtain, a I or II(i) Honours degree in an appropriate subject, and will be expected to register for a higher degree.

The project involves studying the relationship between sex steroids and the endometrium, particularly with respect to implantation, in cases of sub-fertility. The work will be carried out in the Department of Obstetrics and Gynaecology, but the student will also be a member of the Departmentof Biochemistry.

Applicants should write, giving names and addresses of two referees and enclosing a curriculum vitae to Dr P V Maynard, Department of Obstetrics and Gynaecology, University Hospital, Queen's Medical Centre, Nottingham, NG7 2UH. (2903)F

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ASSISTANTSHIPS

QUEEN MARY COLLEGE
University of London
MATERIALS DEPARTMENT

Applications are invited for a

POSTDOCTORAL RESEARCH ASSISTANTSHIP

on a collaborative project between Professor W Bonfield (QMC) and Dr J Bowman (Brunel University) concerned with the development of novel biodegradable composites for an application as bone-fracture fixation plates. The project forms part of an extensive research programme on the biomechanics of bone and bone-replacement materials. A PhD in Materials Science or a relevant discipline is essential and experience in polymer or composite processing and characterisation techniques is desirable. It is intended that the Research Assistant be mainly based at Brunel. Appointment for 2 years in first instance at initial salary in range (under review) £7,190-£8,530 plus £1186 London Allowance. Please apply by letter, enclosing cv and names of 2 referees, to The Senior Personnel Officer, Queen Mary College, Mile End Road, London E1 4NS, by 5 July. (2965)P

UNIVERSITY OF ABERDEEN

DEPARTMENT OF FORESTRY

RESEARCH ASSISTANTSHIP

Applications are invited for a Research Assistantship tenable for three years from October 1984, to study ion exchange at leaf surface as part of a wider study on the impact of acid rain.

The salary will be on the 1B scale for Research and Analogous Staff — £6,310 to £8,530 per annum (under review).

Application forms from the Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 15 July 1984. (2949)P

UNIVERSITY OF OXFORD

DYSON PERRINS LABORATORY

RESEARCH ASSISTANTSHIP

Applications are invited for a post doctoral Research Assistantship (supported by SERC) for studies on the total enantiospecific syntheses of natural products (including sesbanimide and polyhydroxylated alkaloids) from carbohydrates.

The appointment is for one year at this stage from 1 October 1984, or a later mutually agreeable date and is renewable. Salary will be within the range £7,190 - £8,530 pa (Range 1A, Research and Analogous Staff Scales).

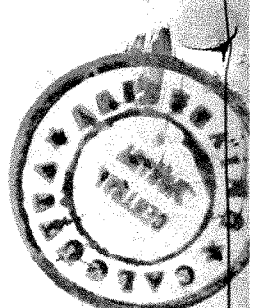
Applicants should send their curriculum vitae, and ask two referees to send letters of recommendation directly to, Dr G W J Fleet, Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford, OX1 3QY as soon as possible and in any event before August 31. (2963)P

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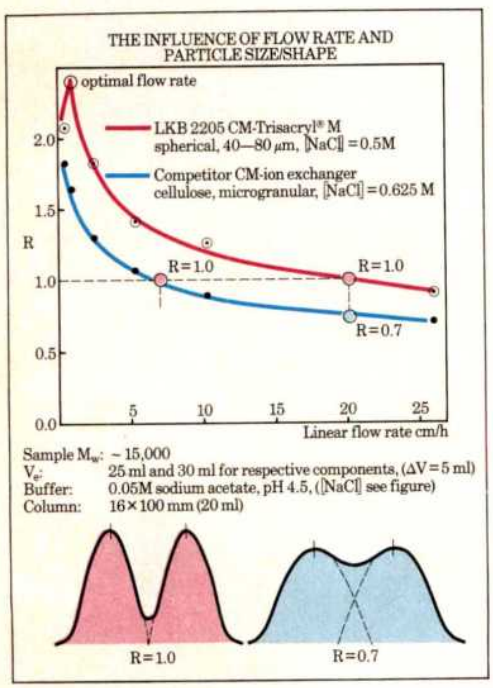
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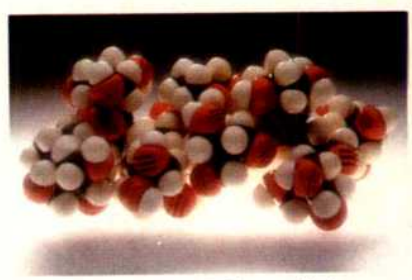


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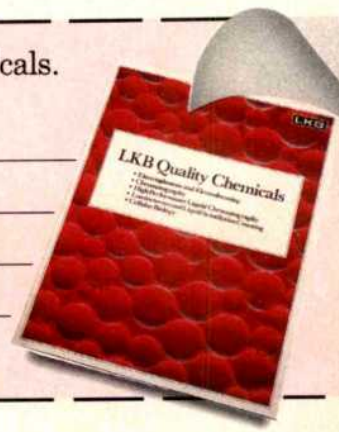
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THE EXHIBITION

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THE CONFERENCE

There is a major conference for analytical science – 'Analyticon' – alongside the exhibition. It caters for scientists and technologists from all branches of industrial science, medical science and related academic disciplines where analytical techniques play an important role. 'Analyticon' is organised by The Scientific Instrument Manufacturers' Association and sponsored by both The Royal Society of Chemistry and The Chromatography Discussion Group. Delegates are expected from more than 20 countries and the scientific programme will cover six main themes: Computers in the laboratory; Chromatography; Molecular Characterisation & Surface Analysis; Elemental Analysis; Clinical Analysis & Bio-sciences; Electroanalytical Chemistry.

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COUNTRY

LABORATORY '84: THE BETTER PLACE TO BE

Recent developments in molecular biology, many of which have been described by their authors in *Nature*, are helping to clarify the causes of cancer. With each passing week, more and more people consider that a full understanding of cancer cannot be long delayed. But the implications for physicians and their patients are still entirely unclear.

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To take part in this important and novel conference, register now and book your rooms (also limited) at the Boston Park Plaza Hotel and Towers.

TO REGISTER FOR THE CONFERENCE: Return the form with your check, credit card authorization or an authorized purchase order. Full 3-day registration fee is $325.

THE SETTING: Boston Park Plaza Hotel and Towers, Boston located at Park Plaza and Arlington Street, just off the famous Boston Public Gardens and Common.

ROOM RESERVATIONS: A large block of rooms has been set aside for attendees, but we do suggest that you reserve your accommodations early, to avoid disappointment. Rooms must be reserved directly with the Hotel, at (617) 426-2000, or 800-225-2008, Telex: 710 321 1088. Be certain to mention your participation in the "Nature Conference", to take advantage of these special lower rates: Singles: $70, $80 & $85; Double: $85, $95 & $100.

CONFERENCE DINNER: The Conference dinner will be held on Tuesday evening, September 11th, in the Imperial Ballroom. TO INSURE SEATING AT THE CONFERENCE DINNER, please include your dinner ticket requirements and payment of $25 per person with your Registration.

POSTER SESSIONS: Please write with a title and a brief synopsis, to the Posters Editor, Nature Publishing Co. 15 E 26 St., N.Y., NY 10010.

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THE MOLECULAR BIOLOGY OF

CANCER

Conference Program
September 10-12, 1984
Boston Park Plaza Hotel

Daily Themes:

DAY 1 - ONCOGENES,
DAY 2 - THE CANCER CELL,
DAY 3 - PROSPECTS/PROBLEMS.

MONDAY, 10 September
Exhibition Viewing: 8:30am — 6:30pm
Recruitment Center: 9:30am — 6:00pm

DAY 1
ONCOGENES AND GROWTH FACTORS — WHAT THEY ARE AND WHAT THEY DO

9:00 — 9:15	**Chairman**
9:15 — 10:00	Oncogenes *R. Weinberg (MIT)*
10:00 — 10:45	Growth factors *M. Spom (NIH)*
11.15 — 12.00	HTLV *R. Gallo (NIH)*
12.00 — 12.30	Clinician's view *S. Broder (NIH)*
2.00 — 4.00	Round table with morning speakers
4.30 — 5.30	The cancer cell *J. Cairns (Harvard)*

DAY 2
TUESDAY, 11 September
Exhibition Viewing: 8:30am — 6:30pm
Recruitment Center: 8:30am — 6:00pm
THE CANCER CELL — HEREDITARY CANCER, TUMOR PROMOTERS AND CARCINOGENESIS

9.00 — 9.15	**Chairman**
9.15 — 10.00	Gene regulation *P. Leder (Harvard)*
10.00 — 10.45	Hereditary cancer *R. White (Utah)*
11.15 — 12.00	Carcinogens and promoters *I.B. Weinstein (Columbia)*
12.00 — 12.30	Clinician's view *F. Gilbert (Mt Sinai)*
2.00 — 4.00	Round table with morning speakers
4:30 — 5:30	*The Pattern of Cancer R. Peto (Oxford)*

WEDNESDAY, 12 September
Exhibition Viewing: 8:30am — 3:00pm
Recruitment Center: 8:30am — 3:00pm

DAY 3
PROSPECTS AND PROBLEMS — DRUG RESISTANCE, MONOCLONAL ANTIBODIES AND NEW DRUGS

9.00 — 9.15	**Chairman**
9.15 — 10.00	Diagnostics and therapeutics *G. Todaro (Oncogen)*
10.00 — 10.45	Immunotoxins *J. Uhr (Texas)*
11.15 — 12.00	Drug resistance *A. Varshavsky (MIT)*
12.00 — 12.30	Clinician's view *J. Gutterman (Houston)*
2.00 — 4.00	Round table with morning speakers
4:00 — 4:45	NCI Program *P. Greenwald (NIH)*
4:45	Closing remarks

TO REGISTER FOR THE CONFERENCE, mail this form with your payment.

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The Molecular Biology of CANCER

I wish to reserve _____ Conference Registration(s) in the names of: *(Please print or type)*

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and _____ Seats at the Tuesday Evening Dinner in the names of:

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Name _____ Title _____

Co./Institution _____

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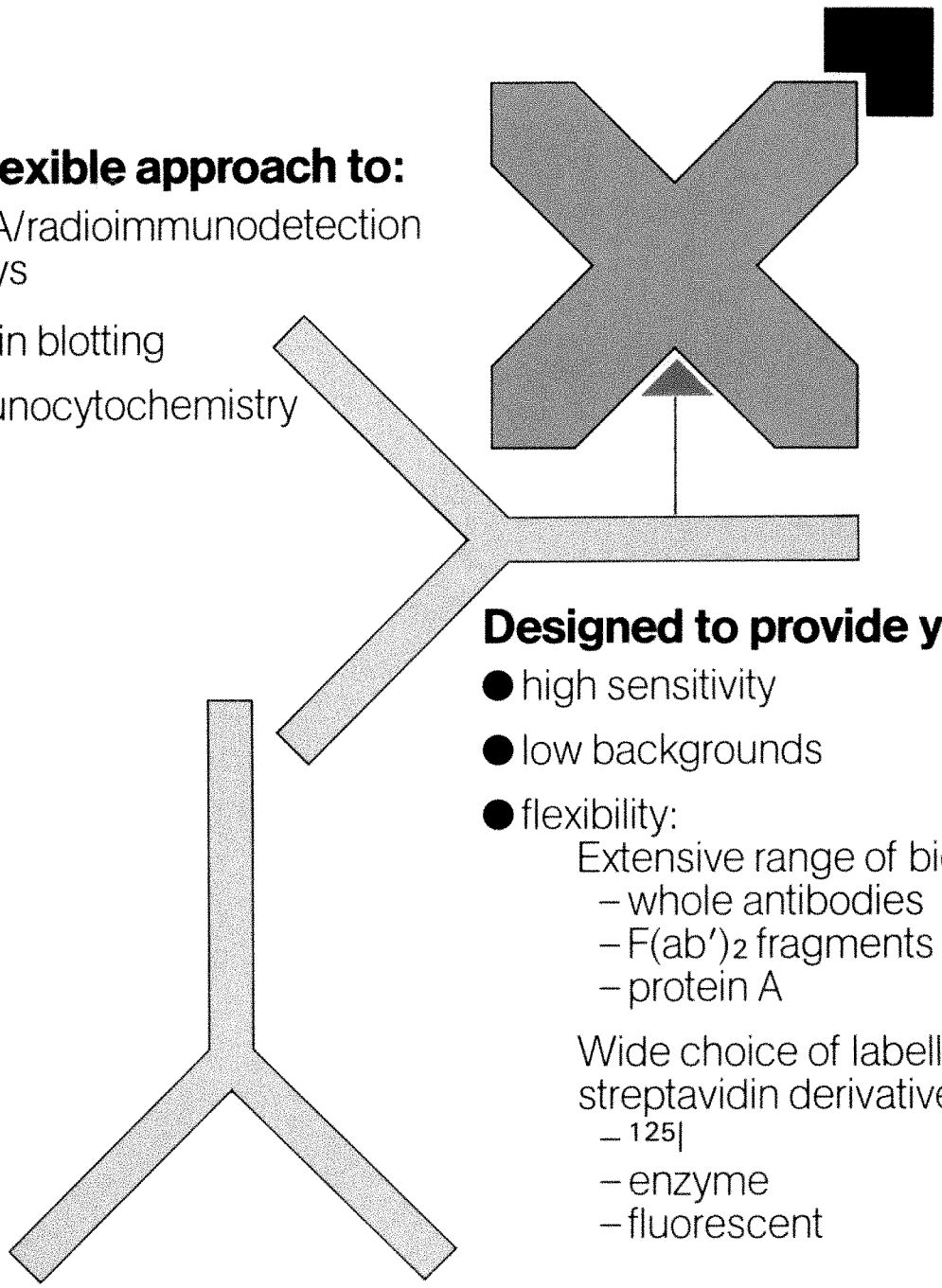
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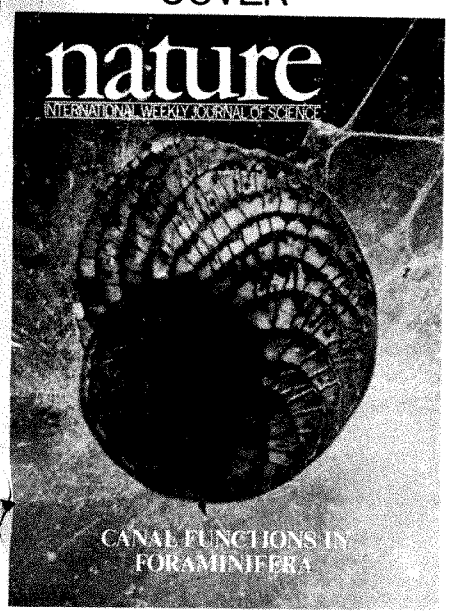
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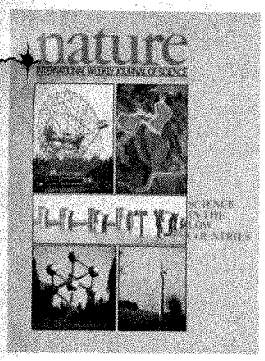
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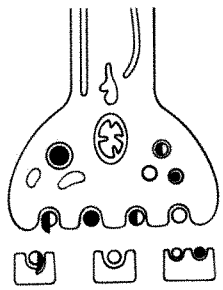
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nature

How to order an East–West thaw

The word in Washington is that the administration is about to try again for better scientific relations with the Soviet Union. The goal should be to put any new initiative on a permanent foundation.

THE tale of how Sir Humphry Davy went on a tour of mainland European laboratories at the height of the Napoleonic Wars is widely regarded as a proof that the international character of science was then more highly regarded than now. That Davy, to whom uxoriousness came late, was also on his honeymoon, makes the journey all the more remarkable. Certainly things are different now, as the near-collapse of scientific relations between the West and the Soviet Union demonstrates all too clearly. Part of the explanation is that research is now recognized to bear on the immediate strategic interests of governments. It also matters that the scientific community everywhere is now dependent on governments for financial support, and thus constrained. But, since the early nineteenth century, procedures by which the movement of people from one country to another is regulated have also been formalized and tightened, partly in response to the flourishing of the technology of travel but also, one suspects, often for its own sake. The time, however, has come to ask that the present parlous condition of East–West relations should not be allowed to continue as it is.

The issue is urgent not merely in its own right but because, by some accounts, President Reagan was earlier this week about to advocate in public a renewed attempt to foster scientific relations between the United States and the Soviet Union. Many outside the Soviet Union will be surprised by this development, not least Dr Frank Press, president of the National Academy of Sciences in Washington. Earlier this summer, Dr Press announced his intention of travelling to the Soviet Union to breathe new life into the largely defunct exchange programme with the Soviet Union. Dr George Keyworth, President Reagan's science adviser, said at the time (see *Nature* 10 May, p.105) that he was "surprised" at this development. But then, with anxiety about the condition of Andrei Sakharov mounting, Dr Press put off his visit. Other academies in the West have responded similarly to recent developments. In London for example, the Royal Society declined a formal invitation to last week's conference in Moscow to celebrate the twenty-fifth anniversary of the Institute of Biochemistry, now run by Academician Y. A. Ovchinnikov. President François Mitterrand, as the world now knows, has chosen a more direct way of dealing with a delicate problem of diplomatic etiquette; last week, in Moscow to seek some basis for continuing East–West negotiations on arms control, he spoke up about Western anxiety about Andrei Sakharov — and had his remarks on the subject censored from *Pravda*'s otherwise complete account of what he had to say.

A sorry tale

The recent deterioration of East–West relations is so familiar and depressing that it hardly bears recitation. Underlying the sorry tale is a sequence of calamitous developments that can be counted as misunderstandings only by the exercise of the utmost charity. The Soviet deployment dating from the mid-1970s of SS20 missiles within striking range of Western Europe broke the spirit but not the letter of the SALT II agreement on strategic arms. Soviet signature in 1975 of the Helsinki agreement within the framework of the Conference on Security and Cooperation in Europe, which among other things provided for a measure of liberality in governments' dealings with their people, did not prevent the arrest of members of the Helsinki monitoring group set up in Moscow. The Soviet intervention in Afghanistan at the end of 1979, ostensibly at the invitation of the government in Kabul, was a more serious setback; the then President Carter gave that as his reason for not sending the SALT II treaty to the Senate for ratification, although by then it was already probable that he would have been given a dusty answer.

Serious interruptions of agreements on the East–West exchange of scientists date, however, from this time. So do the efforts in the United States to restrain the "haemorrhage" of Western technology to the East, with the variously grudging consent of Western Europe and Japan. The exile (to Gor'kii) of Andrei Sakharov, and his subsequent ill-treatment, seems to many to have been the last straw.

Principles

Neither East nor West has dealt rationally with the several sorry events in this unpleasant history. The principles that allowed Humphry Davy to go gallivanting in mainland Europe when France was at war with most other European states seem to have been forgotten. Put simply, they are these. While national governments have necessarily become the chief sources of support for scientific research in all fields, governments have not by that circumstance bought the scientific enterprise, which remains the collective property of its practitioners. And while professional people owe their national governments allegiance of the kind required by the civil law (which varies from place to place), they also owe their profession the duty of acknowledging the international character of what they do. East–West rivalry in military development and in the commercial exploitation of technology, unavoidable at some level, paradoxically must coexist with East–West interdependence in basic science. That is the simple truth that the Pentagon's planners seem only with difficulty to have appreciated in their schemes for restricting visits for foreigners to US laboratories. Exactly the same principle works in the opposite direction, Moscow's puzzlement at the West's indignation over Sakharov notwithstanding.

The translation of these principles into practice should not be as difficult as it is often made to seem by the self-contradictions of governments and the hesitancy of professional people.

If the Soviet Union should send its military forces into Afghanistan on a trumped-up excuse, shoot down a Korean civil aircraft or walk out of negotiations on arms control, that is no reason why the East–West migration of scientists should be constrained. But, naturally, visitors in one direction or the other, being adult electors as well as scientists, will or should be unrestrained in saying what they find objectionable in the policies of their hosts' government. Because of the belief, common in the West and justifiable, that Soviet citizens are not always allowed to judge for themselves what is the truth about political developments, it is inconsistent that exchange programmes should be cancelled or reduced at times when, in a small way, they might contribute precisely to the kind of enlightenment that is supposedly lacking. It is, however, important that these opinions should be advanced only in ways consistent with local civil law and in a manner that does not impede the chief purpose of a visit (which usually, in practice, means privately and not in public). Arguments about the relative durability of communism and capitalism, often entertaining, are irrelevant.

Indignation about the ways in which scientists are dealt with can be accommodated within the framework of seemly East–West relations only with greater difficulty. The Soviet Government's justification of its treatment of Sakharov is logical enough. That a senior physicist, with such important contributions to the Soviet nuclear weapons programme to his credit, should have lapsed into such outspoken criticism of Soviet military and social policies, is regarded as disloyalty bordering on treachery. That view is consistent with the Leninist adaptation to modern times of the feudal Russian doctrine that people are the property of the state. (Soviet apologists for Sakharov's treatment are forever saying that he remains a member of the Soviet academy, with the appropriate salary, but that is beside the point.) What the Soviet Union appears not to understand is the offence which has been given in the West by its treatment of a distinguished if now dissident scientist which, while falling short of outright imprisonment, deprives the scientific community of such contributions as he may still have to make. (It is, on the other hand, unreasonable that Sakharov and his wife should appear to be asking, in a state whose civil laws restrict the freedom of people to move elsewhere — Helsinki notwithstanding — to be allowed medical treatment in the West.)

President Mitterrand seems to have sensed what these circumstances require with his statement in Moscow last week. There is every reason why lesser mortals should, in lesser ways, follow his example. The case is simply that it is a shabby way to treat a distinguished scientist, however troublesome he may have become, to pack him off to a provincial city out of touch with work, friends and colleagues. To say this when asked, and to volunteer the opinion should opportunity arise, need not cause rancour and may in the long run help Sakharov or at least the principles of civility. Better to seize the opportunity than to spurn it, either from embarrassment or in the belief that huffy silence will miraculously be more effective.

That is the most tricky part of the West's case for restoring East–West scientific relations. Let us hope that President Reagan's rumoured intentions materialize — and that the Soviet Union responds positively. But this time, at the umpteenth time of blowing hot, it is also important that the effort should be seriously intended. Nothing so permanently damages East–West relations as a set of formally negotiated agreements that is then vicariously abandoned because of some unrelated happening. So it would be useful to wring from the US Administration an undertaking that if East–West relations are now to be improved, there will be an understanding that they will not in future be put at hazard for political reasons having nothing to do with science. Although the US State Department has been quietly pragmatic in its administration of US–Soviet collaborations even in the past few difficult years, the simplest way of making sure that programmes now agreed continue is to delegate responsibility for them entirely to the National Academy in Washington which, with egg on its face since Dr Press blew hot, then cold, may be needing a little reassurance. □

New exams for old

Britain's plan to reorganize school examinations is sensible, but should be taken further.

THE British Secretary of State for Education and Science, Sir Keith Joseph, last week found himself the unaccustomed object of extravagant praise from all quarters of the British educational establishment. The occasion for this achievement, all the more remarkable in the atmosphere of ill-feeling left by strikes by teachers (over pay) in the past few weeks, was Sir Keith's decision finally to end the archaic and unfair two-tier system of examinations for 16-year-olds. For the past thirty-five years, there has been the "O" (for "ordinary") level of the General Certificate of Education (GCE) and, for twenty years, the Certificate of Secondary Education (CSE) for generally less able students. The notion that the two systems should be merged has been promoted for almost as long as they have co-existed. Five years ago, the present government shelved a plan to merge the systems on the grounds that what it called "standards" would be undermined. Sir Keith's conversion to the educationalists' cause is belated, but none the less welcome on that account. The parable of the prodigal son refers. Will the lessons that have been learned, painfully it appears, now be applied elsewhere in the British educational system?

Sir Keith Joseph's scheme will by 1988 do away with the present jungle of 20 separate examination boards administering an astonishing 19,000 syllabuses for 16-year-olds. The confusion they have entailed has brought the whole examination system into disrepute: it is widely known that (for whatever reasons) the standards of some boards are substantially lower than those of others, even when the qualifications awarded are nominally the same. The boards will now be brought together into five groups to administer a single General Certificate of Secondary Education. Examinations under the new scheme will incorporate differentiated papers or questions that will be assessed to nationally-agreed criteria, thus making them applicable over a much wider ability range than the existing examinations. Students will not therefore have to choose — or, more often, be pushed — between examinations to which, for reasons of parental pride or teachers' convenience, they are not suited. National grade-related criteria now under development will in principle allow a more objective interpretation of examination results — and are indeed essential if the merger is to succeed. In a skilful compromise that seems to have avoided the inevitable accusation that a change will result in lowered standards, Sir Keith has been able to give an assurance that the performance represented by the three highest grades of the set of seven grades in the new examination will correspond to those of the old "O" level.

Why has this apparently obvious rationalization taken so long to decide? The British Government has consistently made clear its position that any integration of GCEs and CSEs would have to wait upon an acceptable agreement on national criteria for syllabuses and assessment procedures, and much effort has been expended towards this end by the examinations boards. Despite a few minor hiccups, such as the minister's reluctance at the outset to accept that a physics examination should include some questions on social and economic issues, the draft criteria have won reasonably wide support. (Even so, its draft criteria for biology at 16 includes no explicit reference to the theory of evolution.) What has been achieved shows that a national consensus on core subject content is feasible.

So will Sir Keith stop now that he has brought about his thoroughgoing revision of the national system of examinations at 16? The existing advanced level examinations ("A" levels), taken by those who choose to study until 18, are just as varied and just as much in need of revision, although their faults are different. In an effort to achieve more uniformity of content, nine examinations boards offering "A" levels have put out a document called *Common Cores at Advanced Level*, but this represents little more than the intersection of the separate syllabuses. And the boards have not yet been able to agree on even this much, with one or other of them declining to accept such-and-such a topic for inclusion. Although the notion that the existing "A" level examinations provide an education that is too narrow for the important years between 16 and 18 is widely accepted, yet, in the present state of anarchy, it is hard to see where a sensible revision in the direction of more generality could start.

The simplest solution would be to abolish this second tier of examinations. But how then would the universities know which school-leavers are best-suited to be students? That would be the academics' cry. There are two answers: provide a university entrance examination (as, for example, in the United States) and abolish the need for school specialization by not expecting most undergraduates to scamper through a first-degree course in just three years. For most British school-leavers who do not go on to further education, some of the time and effort that would be saved by the elimination of over-specialized teaching in secondary schools could be usefully spent in teaching other modern skills, from driving cars to operating computers. □

US space station

NASA negotiates on several fronts

Washington

THE National Aeronautics and Space Administration (NASA) is bracing itself for a complex series of negotiations with Europe, Congress and the space science community before it finalizes plans to build the $8,000 million manned space station called for by President Reagan in his State of the Union message earlier this year.

In Washington last week, a delegation from the European Space Agency (ESA) settled down for what is expected to be a difficult round of talks with NASA about the cost and scope of European participation. NASA, under pressure from Congress and the Office of Management and Budget (OMB) to keep the costs of the station as low as possible, hopes to persuade foreign countries to pay up to 30 per cent of the station's cost.

But the Europeans, who are likely to account for between 15 and 20 per cent of the foreign contribution, are expected to drive a hard bargain.

At a meeting in Naples earlier this month, ESA officials said their member governments wanted to be more than subcontractors; they would be willing to join the project only as full partners able to garner the expertise necessary to launch a station of their own, perhaps a decade or so after the US station is launched in 1992. And to avoid repeating their mistakes with Spacelab, they are insisting on guarantees of equal access to the space station and the right to exploit it commercially.

Foreign participation is not the only difficult question NASA has to resolve, however. Although Congress has agreed to provide NASA with the $150 million it wants in 1985 for preliminary design studies, both houses have signalled that they are not yet fully convinced of the need for the elaborate permanently manned facility planned by the space agency. Bills passed by the Senate and the House of Representatives instruct NASA to set aside several million dollars for "complementary" design studies of a less grandiose "man-tended" facility.

Some congressional champions of the space station objected to the requirement for the complementary studies, arguing that it would send "mixed signals" to the private companies looking at the possibility of investing in manufacturing and research in space. But most members felt it was prudent to keep options open as long as possible, in case budget retrenchment later in the decade shoud leave NASA with a half-completed "'hotel in space".

Meanwhile, NASA is negotiating on a third front, with the US space science community. Although at the outset opposed to the decision, space scientists have wasted no time in joining the planning effort to ensure that the scientific yield from the project is as great as possible. Earlier this month, a newly-established advisory committee on the scientific uses of the space station, under the chairmanship of Dr Peter Banks of Stanford University, recommended that the space station should be accompanied by a flotilla of compact free-flying modular platforms rather than the one or two large unpressurized platforms envisaged by NASA.

A minority of members of the committee is also anxious to persuade NASA to complement the space station, which is to maintain a 400-km east–west orbit at an inclination of 28.5 degrees, with a large free-flying platform in polar orbit. The polar module has been talked about within NASA but is regarded as a prime candidate for cutting in the event of budget difficulties or escalating costs. The platform, regarded as essential by the Earth-observation sciences, is expected to cost at least $800 million.

Peter David

Commercialization of space

Whoever will pay the price?

Washington

US BUSINESS corporations are asking for extensive government subsidies and risk-sharing agreements before agreeing to take up President Reagan's call for the rapid commercialization of space. In Congress last week, leaders of three major aerospace corporations said that, without government help, the costs and risks associated with the exploitation of space are simply too high for it to be worth their while to be involved.

The witnesses, from the Fairchild, Grumman and McDonnell Douglas corporations, are all members of an industry-space commercialization committee which recently submitted a swathe of detailed recommendations to the White House. Although the report has not been published, it is clear that it calls for substantial tax incentives, some form of risk-sharing agreement and enhanced private sector access to the facilities and technical expertise of the National Aeronautics and Space Administration (NASA).

Mr Richard Kline, director of space station operations for the Grumman Aerospace Corporation, told the House of Representatives' subcommittee on space science and applications that the venture capital market rarely finances ventures costing more than $75 million and is too small to support significant investments in space. To help companies reduce their initial investment risk, he said, NASA ought to provide "seed money" for the development of initial projects and reduce the flight of shuttle rides. He also called on NASA to expand its effort in promising areas of basic research such as microgravity and high vacuum environments.

Dr John Townsend, president of the Fairchild Space Company, said a number of complex fiscal and regulatory barriers would have to be removed before the private sector could exploit the full commercial potential of space. The major problem, he said, was that since space is not legally part of the United States, capital ventures based in space do not qualify for the 10 per cent investment tax credit. Congress, he said, should extend to all space ventures the special exemption it granted the commercial satellite industry in 1971.

The White House is expected to consider the private sector's shopping list in conjunction with a separate report on space commercialization drawn up by NASA itself but not yet published. Dr James Beggs, NASA's administrator, admitted that publication had been delayed because of internal disagreements but dodged questions about its specific recommendations. Meanwhile, he made it clear that all was not entirely well with NASA's first great commercial enterprise, its shuttle launch business.

Mr Beggs has two major worries. On the one hand, he is under pressure to reduce the price NASA charges for shuttle flights so that private companies trying to make a profit out of the United States' newly-privatized expendable launch vehicles (ELVs) will be better able to compete. On the other, he is afraid the Department of Defense (DoD), which was originally expected to account for a third of all flights in the 12-year life of the shuttle programme, may decide to divert a large proportion of those flights to its own ELV fleet. While officially describing its retention of ELVs as a "backup", DoD, which has yet to fly its first military payload aboard the shuttle, has been hinting that ELVs offer a cheaper and more efficient answer to its needs.

Only if DoD kept its military payloads for the shuttle, Beggs said, would the shuttle operation be able to recover its direct (but not its research, development and building) costs by 1988 or 1989. That was later than expected, because commercial flights began three years later than planned and the market for the shuttle turned out to be smaller than expected. Nevertheless, he said, NASA would start thinking, towards the end of 1986, about the possibility of selling or giving the shuttle fleet to private companies to manage at a profit.

Peter David

Karl Illmensee

NIH withdraws research grant

Washington

PROFESSOR Karl Illmensee, the embryologist at the University of Geneva accused of falsifying his nuclear manipulation experiments in mice, has now lost his research grant from the National Institutes of Health (NIH). The NIH action follows the finding by an international commission of inquiry in January that errors and discrepancies in Illmensee's records had thrown "grave doubt" upon the validity of the experiments. The commission was unable to substantiate or to dismiss the charges of deliberate fabrication.

Earlier this year, Illmensee's research grant from the Swiss National Fund was revoked. Illmensee has, however, resumed his duties at the University of Geneva.

Illmensee was supported by a $218,000 NIH grant from May 1980 to April 1983. The grant had already been approved for renewal last year when the accusations of data falsification surfaced. On 18 May 1983, Illmensee, at the urging of colleagues who also obtained a now-disputed "confession" from him, wrote to NIH to withdraw a sentence in the grant application that he said had been included by mistake. NIH, concerned about the allegations of fraud and that the withdrawn sentence represented a "major element" of the research proposal, suspended the grant renewal.

After reviewing the commission's findings, NIH decided to terminate the grant application. The decision was taken by NIH director James Wyngaarden on 29 May of this year, but only made public last week after Illmensee had been informed. Wyngaarden also ordered NIH not to award further grants to Illmensee for the same work until he can validate the disputed results. In addition, the commission's findings are to be provided to any NIH review group considering a future grant application from Illmensee.

The NIH officials who studied the commission's report and other available evidence said they could not decide whether the withdrawn sentence in the application had contained "an element of invention", as the commission suggested was possible. But in recommending the actions that Wyngaarden approved, they said that because of "documented deficiencies", the grant should not be made. "In addition, the application was written two years ago and can no longer be considered an up-to-date, state-of-the-art proposal", the NIH officials concluded. The officials also said that no further investigation of the matter by NIH was necessary.

In the withdrawn sentence, Illmensee claimed that a chimaeric mouse had been produced by injecting nuclei from one cell line into mouse eggs of different parentage. Illmensee at first said that the sentence was the result of a typographical mistake made in the haste to complete the application. After being pressed by the commission for an explanation of the actual origin of the chimaeric mouse, Illmensee attributed it to injection experiments involving another cell line; he also sent this revised explanation to NIH. But at the last meeting of the commission, in January, Illmensee said he was no longer certain of that explanation either.

The commission said "it is surprising that Professor Illmensee had to look through his protocols to discover which animal he was referring to in his original NIH application", and concluded that it was impossible to know which "if any" mouse was the chimaera referred to. Illmensee claims that the problem was one of two he has identified.

Neither the commission nor the NIH officials was able to draw any firm conclusions from the accusations of fabrication made by Illmensee's colleagues at Geneva.

Stephen Budiansky

Australian IVF

Orphan embryos

Canberra

LAWYERS in the State of Victoria are at sixes and sevens for lack of legal precedents for dealing with frozen embryos. The issue has arisen because Mrs Elsa Rios and her husband, the parents of two frozen embryos, died in an airplane crash last year, leaving a grown son and an estate estimated at more than $1 million.

Using in vitro fertilization techniques involving donor sperm, an unsuccessful attempt was made by Professor Carl Wood and his colleagues at the Queen Victoria Medical Centre in Melbourne, in June 1981, to implant an embryo in Mrs Rios' womb. She then returned to the United States and the two remaining embryos have been kept frozen ever since. At present, under the common law, an embryo has no rights until it is born, and the State of Victoria will not enact a medically up-to-date legal prescription for questions of custody and inheritance from freeze-thaw embryos until the report of the Waller Committee on the whole subject appears in August.

The Right to Life Association has urged the state's attorney general, Mr Kennan, to appoint a legal guardian to protect the embryos so that they may develop to maturity. Meanwhile, in Los Angeles, twenty-five year old Mr Michael Rios, the son of Mr and Mrs Rios, is reported to have filed for guardianship.

Professor Wood says that the embryos are unlikely to survive because the techniques used at the time they were frozen were not as good as they are now, but no decision will be made about their disposal until after the Waller Committee reports and after correspondence with the executors of the Rios' estate.

Jeffrey Sellar

Childhood vaccine

US plans federal compensation

Washington

IF doctors, pharmaceutical companies and the families of the victims have their way, it will become the job of the US Government to compensate children who suffer damaging reactions to immunization. With the recent announcement by Wyeth Laboratories that it will no longer produce pertussis (whooping cough) vaccine because of the increasing liability burden, pressure has intensified for congressional action to assure that manufacturers will still be willing to produce the vaccines needed and that victims are adequately compensated. Pertussis, polio and measles–mumps–rubella vaccines are all now produced in the United States by single manufacturers.

Lawsuits against the companies are now common. About 60 of the 18 million children immunized each year with the combined diphtheria–pertussis–tetanus vaccine suffer serious long-term brain damage. The usual legal argument of the victims is that the manufacturer or the physician was negligent in failing to inform them of the risks of the vaccine. In some jurisdictions the manufacturer is responsible even when not demonstrably negligent. The companies have refused to discuss publicly the cost of settling these suits.

Under a legislative proposal drafted by the American Academy of Pediatrics and filed by Senator Paula Hawkins (Republican, Florida) and Representative Henry Waxman (Democrat, California), a compensation fund would be created out of surcharges applied to the vaccines. Victims would have the option of suing in the conventional way or of applying to the government for compensation through an administrative procedure. Both the pharmaceutical companies and the Reagan Administration would prefer a bill that prevents victims from suing.

Most of the childhood immunization vaccines used in the United States are purchased in bulk by the Centers for Disease Control (CDC) for distribution to state health authorities. The manufacturers portray their production of the vaccines as a public service, and claim they cannot raise prices to cover the burden of liability without engendering public disfavour. The compensation bill, which would cover all childhood immunizations, is in effect a legally-mandated price increase.

It has been suggested that behind many of the manufacturers' complaints about liability is a dislike of the low profitability of the vaccine business as compared with patentable drugs.

Vaccinations are mandatory before children enter school in the United States. CDC estimate that the pertussis immunization programme prevents 400 deaths per year.

Stephen Budiansky

Japanese legislative logjam

Educational reform held up

Tokyo

PRIME Minister Yasuhiro Nakasone has run into unexpectedly fierce opposition to his plans to set up a special commission to reform Japan's educational system. To push the necessary bill through the Diet, together with the bill for "privatizing" Japan's telecommunications monopoly (see *Nature* **308**, 678 and 763; 1984) and some other controversial bills, Nakasone resorted in mid-May to an extension of the 150-day Diet session by a further 77 days. But opposition parties immediately responded with a boycott of Diet proceedings that lasted until mid-June. Debate has only just begun again, and it is still uncertain whether it will be possible to get the bills passed in the extended Diet session.

Moves to reform the educational system — particularly to prevent the university entrance examination hell — are certainly popular enough among the general public and Diet members. They jib only at Nakasone's insistence that it should contain only members appointed by him without the need for Diet approval ("to ensure neutrality"), that it should carry out its deliberations in private ("to avoid outside pressure") and should report directly to himself rather than to the Diet. Under these conditions, the major opposition party, the Japan Socialist Party, believes that the commission will simply be a rubber stamp for Nakasone's personal views on educational reform.

For the universities, Nakasone's ideas of reform are likely to centre on lessening the importance of the entrance examination. At present, university entrance is simply determined by total test scores in school-leaving examinations. Once entry has been won, very little further is expected of students — giving rise to the view of university as a "leisure land".

Nakasone's opinion, as spelled out in his book *A new theory of conservatism*, is that this should be reversed and that universities should be "easy to enter but difficult to graduate from". He also believes that the university system should be far more flexible, for example by allowing students to transfer credits from one university to another — a move that might well help to break down the overwhelming advantage that Tokyo University graduates have in finding jobs. The problem is that much of Nakasone's plea for more "choice" seems to rely on there being more private education, both at school and university levels; but private universities, according to their own just-released white paper, already see difficulties ahead.

With more than a third of all high-school students now going on to higher education, the number of undergraduates will increase massively between 1986 and 1992 when the second "baby boom generation" leaves school. But after that, university entrants will decline sharply. The private universities point out that they need government support if they are to expand for a six-year period to take the extra students and then contract again. At present, the only way they would be able to cope with extra student numbers is by reducing the quality of education. Nakasone's reforms are thus becoming entangled with the level of state support — now declining — for private universities. As it is believed that Nakasone wants to appoint the present head of the private Universities Federation to leadership of the reform commission, the state universities believe that the whole reform plan might end up robbing them of funds.

The most vociferous opposition to the establishment of the commission has come, however, from the school-teachers' union *Nikkyoso*. The union claims a membership of 71 per cent of the primary and junior high-school teachers (though the Ministry of Education says the real figure is 51 per cent) and is left-wing in its views. At its annual convention last week — which required the presence of 2,500 riot police to prevent disruption by right-wing extremists as in the past two years— it decided to have nothing at all to do with the commission. Nakasone had been hoping that he could win support by having a *Nikkyoso* representative on the commission.

The union is naturally worried by the emphasis Nakasone places on "moral education". In March for example, he said in the Diet that "patriotism and the traditional Japanese respect for one's parents should be taught at school". To the left wing this seems too much like a return to the nationalist education that existed before the Second World War, and its members claim that signs of a swing to the right can be seen already.

The contents of school textbooks seem to be being more tightly controlled this year and the Ministry of Education has been accused of using its powers to dilute the facts of the darker side of Japanese history.

For Nakasone, much more rests on the successful passage of the bill than his own personal interest in educational reform. In November, he has to face the bi-annual elections for the Liberal Democratic Party leadership, success in which assures that he continues as prime minister. Despite his popularity abroad and his emergence as Japan's first international statesman, his performance at home has been lacklustre. He will be judged by his fellow party members mainly by his ability to handle the Diet, but in this session he has failed to get a single major bill through (although a measure to increase Diet members' salaries passed with great speed). As well as the educational and telecommunications bills, bills to reform the health system and the electoral system are waiting.

Alun Anderson

Tibetan geology

UK–Chinese joint venture

A COLLABORATIVE research project on the geology of the Tibetan Plateau has been announced by the Royal Society of London and the Chinese Academy of Sciences. It will run for two years from 1 July, with the actual fieldwork taking place in the summer of 1985. Fourteen Chinese scientists and twelve from the West (largely from the United Kingdom), under the leadership of Professors Chang Cheng-Fa, R.M. Shackleton and J.F. Dewey, will spend eight weeks covering more than 1,000 km in perhaps the most inaccessible part of the world. The estimated cost of approximately £350,000 is being shared roughly equally between the two academies, with the Chinese providing the back-up for the expedition.

Scientifically, the project is complementary to the joint French–Chinese investigations in southern Tibet in 1980–83, as a result of which earth scientists now have a better understanding of the mechanisms of formation of the Himalayas and other linear mountain belts. In contrast, the means by which vast areas of relatively undeformed rocks can be uplifted (to an average elevation of 5 km in the case of the Tibetan Plateau) are still obscure, in spite of theories based largely on consideration of the rheology of the Earth's crust. The Anglo-Chinese project should provide the hard facts against which these theories can be tested.

The project involves a 1,000-km traverse from Lhasa to Golmud, on the northern edge of the Tibetan Plateau. Geologically, Lhasa sits on a block representing the southernmost part of Asia, at the junction with the Indian block to the south. North of the Lhasa block, the geology is poorly known, although a number of crustal units have been recognized. Only painstaking fieldwork, building on the reconnaissance surveys of the host nation, will clarify the relationship between these units.

The major results of the French–Chinese work relate to the past 20 million years, although reports of the second expedition in 1981 (see *Nature* **307**, 17–36; 1984) suggested that Tibet may consist of pieces broken off Gondwanaland, which moved north in advance of India, perhaps in successive waves, and eventually collided with Eurasia. The British aim to delve deeper into the past, using predominantly palaeomagnetic and faunal studies, to the earliest separation 150 million years ago in the late Jurassic. The detailed histories of these continental fragments may be better known as a result of the new project.

Negotiations started in 1981 and the programme is regarded as a landmark in the continuing expansion of collaborative programmes between the Royal Society and the Chinese Academy.

Peter Gambles

Dutch nuclear issues

Decisions later

Waalre, The Netherlands

THE Dutch preoccupation with nuclear energy, military and peaceful, will continue for at least another eighteen months. That is the effect of the decision of the Dutch Parliament on 13 June that a final decision on the siting of US cruise missiles in the Netherlands will be made only at the end of 1985. The Netherlands is the only country out of step with the policy of the North Atlantic Treaty Organization (NATO) in this respect.

Never has a subject been discussed so feverishly in the Netherlands. Only the political parties on the right (conservatives and four smaller parties) are in favour of deployment of the missiles, but, according to opinion polls, voters are against the siting plan. On the left, the socialists and three small parties are against, as are the great majority of their voters. The role of the Christian democrats, which together with the conservatives form the government, is especially confusing. Their voters are sharply divided, and they are under heavy pressure from the churches which in advance of 13 June issued declarations asking the government not to install the missiles and in the process offended some church people who hold that the declarations were "too political".

Similarly, the development of civil nuclear power in the Netherlands is on ice, with more than half of the population against it. There are two small nuclear plants (of 60 and 470 MW) now operating, and ten years ago the government proposed building three 1,000-MW plants. But that plan was shelved after a year and a half because of the strength of the opposition, unabated by the oil crisis. But discussion continues, with the churches and the environmental movement playing a prominent part.

Earlier this year (see *Nature* **307**, 405; 1984), it seemed the issue had been settled by the report of the officially organized public consultation which in the previous two years had gathered evidence for its conclusion that further nuclear plants are now necessary and that combined heat and power schemes, in industry and domestic applications as well as wind power, should replace obsolete plants in the 1990s. But now it seems that the government is brooding on plans to expand nuclear capacity by 4,000 MW, and leading newspapers have been complaining that this overrides the democratic consultation the government itself had organized.

The government's plans for nuclear power will in fact be published in September but meanwhile the minister of economic affairs, Gijs Van Aardenne, who was unenthusiastic about the report on nuclear power published in January, does not conceal his enthusiasm for further development, while Prime Minister Ruud Lubbers, in 1974 the minister of economic affairs who put forward the earlier nuclear power plan, is also a supporter.

So the months ahead will be another test for democracy in the Netherlands. Although the debate about the missiles will continue for 18 months, it is barely conceivable that the Netherlands will not follow its NATO partners, despite five years of public demonstrations and social unrest. And the development of nuclear power may follow the same route.

Casper Schuuring

UK science budget

Money needed

THE British Government's advisers on the share-out of the civil science budget have warned Members of Parliament of the dire consequences for the nation's research enterprise unless greater provision is made for science over the next decade.

The House of Commons Select Committee on Education, Science and the Arts, which has started an inquiry into the science budget, heard last week from the Advisory Board for the Research Councils (ABRC), represented by its chairman, Sir David Philips, Dr Walter Bodmer, director of research of the Imperial Cancer Research Fund, and Dr Derek Roberts of General Electric Company Ltd. On the basis of published government expenditure estimates, ABRC predicts that the real value of scientific research is set to decrease by 25 per cent over the next decade.

This stark prediction arises from a number of effects that diminish the value of the research budget, which at first sight is planned to remain roughly constant in real terms. Firstly, according to ABRC, the science vote has had largely to compensate for an 11 per cent cut in recent years to university support of science from the University Grants Committee. Secondly, the Treasury's assumed annual cash increase for the science vote, which decreases from 3 per cent per year to 2.5 per cent per year over the next decade, is fully 2 per cent less than the Treasury's estimated annual increase in the retail price index. This alone would produce a decrease of 17 per cent over the 10 years in real terms.

On top of this, there is the problem of superannuation payments for the increasing number of retired research council staff. And the perennial problem of subscriptions to international organizations still remains. ABRC members emphasized last week their conviction that science had lost out in recent years in relation to other publicly supported activities, while the increasing sophistication of scientific equipment has meant that the same amount of money bought less. And they were disturbed that the government's published expenditure plans include no special reference to the value of science in the economy.

Tim Beardsley

Acid rain

UK in minority of one

THE British reputation for waywardness in the eyes of many Europeans seems unlikely to be improved this week at the World Conference on the Environment, taking place in Munich at the behest of the government of West Germany to discuss ways of combating atmospheric pollution. At the opening session on Monday, Dr Martin Holdgate, chief scientist of the British Department of the Environment, declined to give any firm commitment to reduce British emissions of sulphur dioxide, saying only that a range of measures was being considered that would allow Britain to reduce emissions "in a cost-effective way".

In Germany, acute concern over damage to forests is leading to pressure for drastic reductions in acidic emissions, chiefly sulphur dioxide from power stations and nitrogen oxides. But Dr Derek Pooley, chief scientist at Britain's Department of Energy, said last week he was "convinced that sulphur dioxide was not the major culprit in causing damage to forests".

A major study on acid deposition published last week by the department's energy technology support unit emphasizes the uncertainties. Forest damage appears not to correlate well with sulphur dioxide concentration when dry deposition from local sources is excluded. But above an altitude of 600 metres, which is where most of the damage occurs, photochemically produced ozone can reach sustained and high concentrations. Although no mechanism for ozone damage is known, one suggestion made in the Department of Energy's study is that ozone causes damage to leaf cuticle, which in turn allows leaching of magnesium as the *p*H falls. But Dr Bill Binns of the Forestry Commission, who has made

Cost of impure mice

Washington

A UNIVERSITY of Wisconsin researcher has won an out-of-court settlement with Charles River Breeding Laboratories over a mix-up of mice that the researcher said caused her to waste a year's work and set back her career. Dr Brenda Kahan will receive an undisclosed monetary settlement; in addition, the University of Wisconsin will receive $40,000 from Charles River as compensation for research and overhead expenses of the invalidated research. Two-thirds of that $40,000 will support Kahan's research.

Charles River, a major supplier of laboratory mice, shipped large numbers of genetically impure mice to researchers who believed they were getting a pure strain. The company says it has now corrected the problem. Stephen Budiansky

several studies of forest damage in West Germany, is not so quick to rule out a role for sulphur dioxide. He emphasizes that "occult" precipitation — fog and mist — can be much more acidic than rain and may yet play a part, perhaps in conjunction with other stress factors.

If there is still mystery over the mechanism of damage to trees, the mechanism of damage to fish in fresh water is rather better understood. It seems that elevated levels of inorganic aluminium may be a major cause of mortality. But the chemical changes that will occur in a body of water subjected to acid deposition within its catchment area are greatly affected by the nature of the soil and bedrock, as well as by the type of vegetation present. It is clear that coniferous trees, in particular, can efficiently filter out acids from the air and release these in solution at the next rainfall. The Forestry Commission in Britain is already investigating management practices that may reduce acid release into streams.

Because of the multitude of factors that affect acid depositions, the precise attribution of blame is difficult or impossible. Motor vehicles, for example, along with power stations may be (indirectly) a major source of ozone, through their emissions of nitrogen oxides. But the British contingent at Munich this week will doubtless be able to make much of new estimates published by the Department of Energy of the proportion of British-emitted sulphur dioxide that finds its way to other countries: 3.6 per cent to West Germany, 4.4 per cent to Norway and Sweden combined, while almost 30 per cent stays in Britain.

While the British Department of Energy harks on the quantitative uncertainties of acid deposition, the Nature Conservancy Council, which also last week published its views on the matter, takes a somewhat different line. It argues that acidification reduces species diversity in aquatic systems and that the only long-term solution to the problem is therefore to reduce emissions of sulphur and nitrogen oxides. And it is opposed to the policy of liming affected waters on the grounds that the practice will in the long term bring its own undesirable effects.

The British Government seems likely to find itself increasingly isolated over acid emissions. The government is commited to reply before the end of the current parliamentary session to the latest report of the Royal Commission on Environmental Pollution, which urged that the Central Electricity Generating Board should introduce emission abatement measures on a pilot basis. And the Select Committee on the Environment of the House of Commons is shortly to report on the results of its inquiry into "acid rain", which, it may be guessed, will not take a complacent view. It seems likely that political pressure will before long force some government concessions. **Tim Beardsley**

Pesticides

New chemicals under fire

THE latest returns in a two-year survey of the British barn owl population suggest an even greater population decline than had been feared. Ten years ago there were some 4,500–9,000 pairs in England, Scotland and Wales and now, after 18 months of the "Barn Owl Survey" organized by the Hawk Trust, the indications are that the overall decline has been at least 10 per cent, with much greater reductions in many areas.

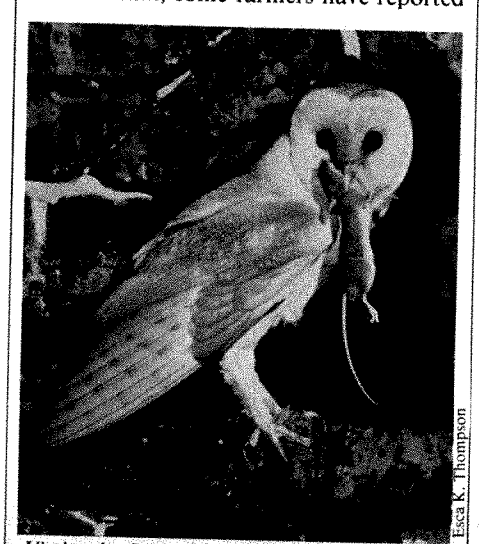
The only areas where there has been little decline are in Scotland, where the Forestry Commission's activities have provided some additional refuge for the dwindling population. The population estimates, however, are becoming increasingly inexact as the numbers involved get smaller.

The continued decline of the barn owl is thought, says Colin Shawyer of the Hawk Trust, to be a result of the use of new rodenticides by British farmers. Difenacoum and brodifacoum have been growing in popularity in the past few years, steadily replacing warfarin, which is known to be relatively non-toxic to species other than rodents. Brodifacoum is thought to be especially toxic to birds and for that reason has not been given full approval by the Ministry of Agriculture, Fisheries and Food under the Pesticides Safety Precautions Scheme.

Some of the evidence for the effects of the new pesticides comes from Malaysia. On some oil palm plantations, the combination of warfarin and the wild barn owls, encouraged by the provision of nestboxes on poles throughout the plantations, has effectively controlled severe rat infestations. But even on estates where there has been no evidence of warfarin resistance, the new pesticides have been tried. And, says the Hawk Trust, barn owl numbers declined so rapidly that the estate managers went back to the old methods.

In Britain, some farmers have reported to the Hawk Trust that barn owls seemed to disappear from their farms soon after they started using the "second generation" rodent baits. And a veterinary surgeon reported that a barn owl thought to be dying from the effects of difenacoum poisoning was successfully treated by the vitamin K antidote to the pesticide.

Much of the evidence on pesticide toxicity is circumstantial, the Hawk Trust concedes. So the urgent need is for more research on the effects of the new chemicals on non-target species. **Charles Wenz**



Esca K. Thompson

Victims both — a barn owl and prey.

Irreproducing ibis

Tokyo

EFFORTS to save Japan's *Nipponia Nippon* (the Japanese crested ibis or "toki") seem doomed to failure after a romance between two of the remaining birds — Kin (the female) and Midori (the male) — ended in a mid-mating season fight. As Kin is now, in human terms, around 80 years old, the chances of a successful mating next year are thought to be close to nil, although hormones are to be administered in the hope of keeping her in breeding condition.

The toki, a large white bird with a characteristic long downwardly curved black bill and a red face, was a common sight in wet paddy fields and ponds throughout Japan and parts of east Asia as recently as one hundred years ago. But a catastrophic decline in their numbers this century led to their being given special status in Japan in 1934. The war prevented any serious conservation measures, however, and by 1953 only 31 birds remained.

In 1966, special measures were begun but numbers continued to decline so, in 1981, the remaining five wild birds and one captive bird were removed to a breeding centre on the island of Sado — where in bygone days troublesome cadet branches of the imperial family were exiled so that their lines might disappear. Two birds died but hopes rose in 1983 when two of the remaining four birds paired. The female died, however, from a blocked fallopian tube shortly before she laid her eggs.

This year, the romance between Kin and Midori was followed closely but to no avail. As well as being in ripe old age, Kin, having been reared in captivity, apparently did not take too well to Midori, who had led a life in the wild until 1981.

The Sado Island Toki Centre is now to advance research on artificial insemination as fast as possible, but researchers doubt whether practical methods can be developed in time. Hopes for saving *Nipponia Nippon* from extinction now rest largely with the Chinese. In 1980, seven birds were found in China, raising the known numbers there to fourteen — the same as the number remaining in Japan. There have been occasional rumours of sightings in Korea and the southeastern Soviet Union, but none has been confirmed. Alun Anderson

Science and research in India

SIR — You say in your study of science in India that there is no dearth of funds. That is true. Organizations such as ICMR, CSIR, DST and UGC have enormous funds. But how much of this money goes to deserving people? Who, in any case, is deserving? In my opinion, those who deserve research funds are those who have worked in a specific area. Yet as things are, persons who have never seen tribes, never travelled in forest areas and have not worked on any aspect of anthropometry, haemoglobinopathies or population genetics may be allowed to spend 5 million rupees at the expense of investigators with relevant experience.

Last year, I asked a senior official of a major funding agency about this practice. His chilling answer was that "we do not wish even to look at projects costing less than three lacs" (300,000 rupees). He further added that nothing can be done in science these days with one or two lacs of rupees. The truth is that there appears to be no remedy.

In India, there are few first-rate academic institutions when compared with the enormous number of students graduating every year. The general decline of students' interest in higher studies and the mushroom growth of colleges everywhere are unrecognized impediments to India's progress. The tolerance and even the cultivation of corrupt practices in the name of democracy is rampant in all universities and colleges, and in the publication of research work, as discussed in your report.

In my view, four out of five of our universities do not have enough money to support the basic necessities of modern science education, despite modest support from the University Grants Commission. The first reason is the apathy of state governments towards higher education, the second, the outlook of the bureaucrats who control events and, the third, the incompetence of the vice-chancellors. Nowhere in the world have vice-chancellors such an uncertain future (they are replaced so often) and so little power. Even those with vision and ability can be quickly cowed by an under-secretary of the state government on any trivial matter. The exchange and extraction of mutual benefits is rife in the entire university education system. So I would modify *Nature*'s slogan "Excellence in the midst of poverty" to "Excellence against all odds".

My twenty years of teaching experience lead me to conclude that most talent is distracted from research. Many faculty positions, research scholarships and senior fellowships are lying vacant and at least 60 per cent of research scholars leave research fellowships before completion. Nobody wants to acknowledge that part of the reason is the victory of incompetence over ability, mediocrity over genius. In my view, corrupt politicians and officials poison social progress only slowly, but corrupt teachers and particularly scientists can spoil generations. No nation should aim at nurturing mediocrity, whatever its national beliefs.

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SIR — In my experience Indian scientists have the zeal and energy to succeed, but are handicapped by limited facilities and experience. The majority of institutes in India are sub-standard and producing graduates with limited practical knowledge, as boldly accepted by Dr Subramanian (*Nature* **307**, 206; 1984). One reason for this is that institutes away from the centre suffer step-mother treatment. The centre and the University Grants Commission, the two main funding authorities, are too easily influenced by individuals heading the institutes governed by the centre, or by centrally located organizations who can approach the funding authority directly.

To obtain a research grant in India, one has to be not scientifically orientated, but politically motivated. The system brings non-scientifically minded individuals to the top who are against the return of their scientific compatriots from abroad, and cannot see their local colleagues rising quickly unless they share the credit for their work. Dr Balasubramanian (*Nature* **307**, 321; 1984), sitting in one of the central institutes, has perhaps never had a chance to see establishments which lack the basic necessities to teach or initiate scientific research.

The main problem facing the plan to site an international centre in India (*Nature* **305**, 350; 1983) is that of supply of basic chemicals. Local products are not up to standard, and to import foreign goods, even after going through the red tape, one may have to pay 110 per cent customs duty on essential consumables of research. Sometimes it takes days to get supplies through customs.

Admittedly, India has its nuclear and space programmes to show the world but I do not agree with the claims of Dr Balasubramanian, who is perhaps unaware of the high prevalence of leprosy, tuberculosis and other communicable diseases in the very state where he is working. India must concentrate on educating society and research programmes should aim directly to reduce such human suffering. Many expatriate Indian scientists may wish to go back and the idea of a technology city is very rosy to them, but unless this city is controlled by a scientist with special powers to cut through the bureaucracy of Indian authorities, the city will be a failure. To raise the existing standards of Indian research, direct communication and collaboration with international scientists should be promoted.

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SIR — In your otherwise interesting survey, you were not able to give much time to the decaying but historical city of Calcutta, the first capital of British India, and the cradle of modern science in India. You rightly mentioned the late Sir J.C. Bose, the first Indian physicist FRS (1920) and his Bose Institute, founded in 1917 on the model of the Royal Institution of Great Britain. You also mentioned the late Professor S.N. Bose, FRS (of Bose–Einstein statistics) and his institute. But you omitted to mention a giant of Indian science, the late Professor M.N. Saha, FRS, a class-mate of the late S.N. Bose. It was after their joint English translation of Einstein's relativity papers in book form (published by Calcutta University) that Professor Bose was able to continue his work on his chosen subject while at Dacca University.

Professor Saha (the third Indian physicist FRS elected in 1927) made his impact on the world of astrophysics through his famous thermal ionization work in 1920, and became the first Khaira Professor of Physics, Calcutta University in 1921. After a spell at Allahabad University on his return to Calcutta in 1938 Professor Saha installed the first Indian cyclotron at what is now the Saha Institute of Nuclear Physics, inaugurated by the late Madame Joliot-Curie in 1951 with Saha as the founder-director. This institute also built the first electron microscope in India, and now has a thriving biophysics division.

In the premises of the Calcutta University Science College another great Indian scientist, the late Professor S.K. Mitra, FRS, established the Institute of Radio Physics and Electronics.

Third, at the Presidency College, Calcutta, the late Professor P.C. Mahalanobis built the nucleus of the Indian Statistical Institute. It was at Presidency College that the first cloud chamber for cosmic ray studies was constructed by Professor Rajen Sengupta, then a student of the late P.M.S. Blackett.

Finally, at Calcutta today the most active centre for scientific research is the Centre of Advanced Study on Cell and Chromosome Research, directed by Professor A.K. Sharma (in collaboration with his wife Mrs Archana Sharma), at the Department of Botany, Calcutta University. Professor Sharma's new technique for the study of the physical and chemical nature of chromosomes has been widely adopted all over the world.

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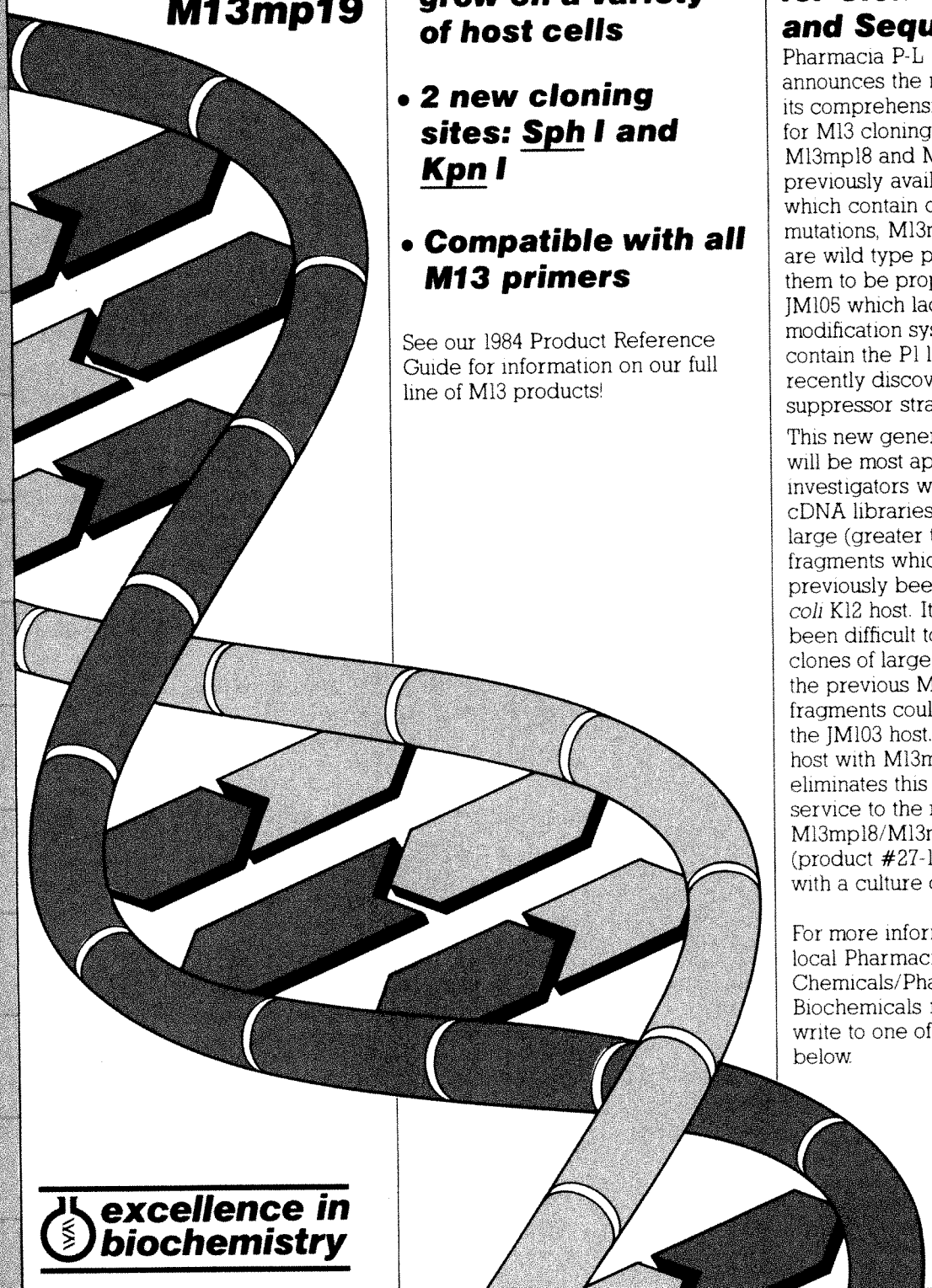
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Lights hidden under bushels

Geochemists are not alone in failing to make clear the elementary assumptions on which they work. The result is that what they do is under-appreciated.

GEOCHEMISTS, who have a great many achievements to boast of in the past few years, are sometimes their own worst enemies. That is what many innocent readers will conclude from a casual inspection of the rush of articles with which the geophysical literature is now spattered, and which purport to show how, for example, the measurement of neodymium isotopes in some sample of rock can shed light on the past history of the material. The articles on pages 753 and 773, singled out perhaps unfairly as representative of their kind by the mere accident that they are included in this issue, show how unforgiving of their readers' patience the geochemists have become. (If they should protest that they are not alone among specialists in making the assumption that everybody who matters knows what they intend to say, they are merely making the more dangerous assumption that only fellow specialists are likely to be interested).

The underlying difficulty is common in new fields of investigation, perhaps especially in those almost certain to become important. It is then tempting for those with new things to say to work on the principle that the assumptions with which they live day by day are widely appreciated. If indeed, with the passage of time, the novel branch of geochemistry based on the analysis of terrestrial (and other) rocks for the radioactive decay products of rare but long-lived components of the Solar System turns out to be as important as it promises, the result will be that the general readership of the literature will know what the assumptions are. This, to a large extent, is what has happened in the previously daunting field of nucleic acid chemistry, where it is no longer necessary for people to spell out the simple fact that DNA molecules are constructed from four distinct subunits, for example. But if the new geochemistry should be only of middling importance, is it destined to remain more or less a closed book?

That would be, to say the least of it, a pity, which is why, at least for a little while, geochemists should be a little less austere in the way they describe what they have done. It is not that the field is all that unfamiliar. Indeed, it resembles in many ways the pioneering work of F. W. Soddy, in the first decade of this century, in seeking to determine the age of the Earth from the radioactive decay of uranium and thorium.

The essence of that task, successfully completed in the 1930s, is that two principal isotopes of uranium (with mass numbers 238 and 235) ultimately yield as decay products two isotopes of lead (with mass numbers of 206 and 207 respectively). Thorium-232, on the other hand, finishes up as lead-208.

What this implies is that it is possible to infer the length of time for which a rock containing uranium has been undisturbed by a sufficiently accurate measurement of the amount of, say, uranium-238 and of the abundance of lead-206 relative to that of some other isotope of lead known not to be the product of radioactive decay. (Lead-204 is the common standard.) For then the lead-206 found in a sample of the rock will represent both that formed by the decay of uranium-238 and that present at the outset. If the primaeval abundance of lead-206 relative to lead-204 is known (as, for example, from the analysis of rocks containing lead but no uranium), it requires merely simple subtraction to discover how much lead-206 has been formed by the decay of uranium-238.

The techniques now being exploited with such profit by the geochemists are in principle no different. Neodymium-143 is formed by the alpha-decay of samarium-147 (with a half-life of more than 100,000 million years) and strontium-87 from rubidium-87 by beta-decay (with a half-life of nearly 50,000 million years). Relative to other isotopes of neodymium and strontium, the excess amounts of the radiogenic daughter elements in some sample of rock are simply related to the amounts of the radioactive isotopes originally present and to the length of time which has elapsed. The conventional standards for comparison are the isotopes neodymium-144 and strontium-86 respectively.

Much of the confusion that attends the use of these techniques in geophysics stems from the recognition, in itself a discovery of great importance, that the elements involved, the radioactive parents and their daughters, have beeen repartitioned among the several components of the Earth's layer structure during geological history, not all of it Archaean. Both rubidium and strontium, as chemical elements, are more abundant in continental crust than in the mantle from which the continents were formed, but the relative concentration of rubidium is greater than that of strontium (by factors of perhaps 70 and 20 respectively). With samarium and neodymium, by contrast, the daughter tends to be more highly concentrated than the parent.

Chemists are quick to point out that this redistribution of material is largely a function of the radii of the ions of the elements involved. (By chance, uranium and lead are concentrated in the continental crust relative to the mantle by roughly the same degree.) Whatever the mechanism of this process, what emerges is that the abundance of strontium-87 (relative to strontium-86) is greater in the continental crust than in the mantle, while the abundance of neodymium-143 (relative to neodymium-144) is less than in the mantle.

The interpretation of the ratios actually measured is complicated by the difficulty of knowing the full history of the material concerned. In attempts to infer the age of the Earth from measured abundances of uranium isotopes and the corresponding isotopes of lead, it is sufficient to search for the oldest rocks and to compare the results of abundance measurements with those obtained from meteorites. By contrast, the chief utility of the use of the newly-measurable isotopes is that they may be used to trace the comparatively recent history of the Earth's surface layers, continental crust and ocean floor alike, in which case there can be no fixed assumptions about the abundance of the isotopes in the starting material.

This does not, however, prevent practitioners from talking of "anomalies". The reference point is usually some notional composition of primaeval material but is hardly crucial, since only differences of abundance bear on the processes by which material has most recently been repartitioned. Eventually it should also be possible to construct a kind of historical record of the average abundance in the mantle of the isotopes involved, at least if the mantle is crudely homogeneous.

All this, of course, is commonplace among geochemists. But now and again, it would do no harm if they were to remind their readers that neodymium-143 is the product of the radioactive decay of samarium-147, or that lead-206 is the product of uranium-238. Certainly they should be more forgiving of their readers' almost certain ignorance of the importance of these isotopes as tracers of the fractionation of terrestrial material. To fail to make these concessions is to underplay an exciting field.

John Maddox

Fisheries

Genetics, dynamics and politics

from John H. Lawton and Robert M. May

MARINE fish stocks, broadly interpreted to include such taxonomically disparate beasts as whales, shrimp, crabs, shellfish and squid, together with fish in a strictly zoological sense, constitute one of the world's great biological resources. Attempts to understand, conserve and exploit resources confront marine biologists with a number of intriguing theoretical and practical problems, often made more difficult by political and economic constraints operating on the fishery. A recent Dahlem Conference* grappled with a range of such problems.

Some of the biological problems confronting fisheries managers have implications for evolutionary biology and ecology far outside their primary concerns of maximizing profits and minimizing the risks of overfishing. Two, namely fishing as a major selective force acting on the genetic composition of fish stocks and the problem of how best to construct tractable models of complex multi-species fisheries, seem particularly important.

Often, though by no means always, fishing a previously unexploited stock leads to faster growth and earlier breeding of the surviving population[1]. Predictions of yield, profits and the population's capacity to withstand exploitation hinge on a sound understanding of such changes in demography under exploitation. The changes can happen quickly, and have generally be attributed to the fact that as stocks are fished down, food supplies per individual increase, leading to faster growth and earlier maturity. However, an explanation based on such density-dependent effects in the population dynamics is not the only possibility; the response could also be genetic; if, as seems likely, growth rates and age of maturity are genetically determined, and there is heritable variation for these traits within the population, then fishing represents a massive novel selection on the population. Individuals that grow faster and/or mature earlier may be at an advantage in the fished population because, other things being equal, they are more likely to reproduce before being caught. Other mechanisms possibly responsible for the observed demographic changes include alteration in environmental variables that more or less accidentally coincide with changes in fishing intensity, or even that apparent changes may be artefacts of the way the data are gathered.

Although it is difficult to distinguish genetic from other explanations for changes in the vital rates of exploited fish stocks, useful tests may come from situations where fishing, paradoxically, leads to a reduction in growth rates and to an increase in the mean age of fish. This apparently happened in the case of the Lesser Slave Lake Whitefish, *Coregonus clupeaformis*[2]; such changes are not easily accounted for by responses in population dynamics to fishing, but may be due to heavy selection of gill nets on large rapidly-growing genetic variants in a species where reproduction is strictly determined by age and not by size[2].



In most populations we might expect to see both population-dynamic and genetic responses to fishing, working either in concert or in opposition to one another to influence the demography of the exploited population. The consequences are important for managers wishing to predict the long-term dynamics of fish. But over and above all these practical considerations, evolutionary biologists, interested in life-history strategies[3], should pay more attention to the possibility that fish life-histories can evolve in response to fishing.

A second area where essentially practical problems of fisheries management may have wide theoretical repercussions centres on how best to construct tractable models of complex multi-species communities. Not unexpectedly, the fisheries that are most rich in species are in tropical waters. For example, off the Senegal–Mauritanian coast as many as 174 species are involved in the demersal fishery; a single trawl haul can contain 20–50 species. Official statistics for this area tend to group catches into commercial categories, but still 48 species are identified in the records for Senegal[4]. Classical fisheries models, based on single species, are obviously no use under these circumstances. However, detailed simulation models involving all the commercial species and their food supplies are not a solution either. Even if the data were available, the sensitivity of the very complex models to errors inherent in estimating the snowstorm of parameters necessary for their construction makes them unworkable.

Simplified models are much less sensitive to parameter variation, but the problem is how to simplify them. Grouping species into manageable sets could be done in several ways, for example taxonomically, or by habitat, or by commercial value. At the moment, there are no reliable theoretical rules to guide such endeavours, and the best solution must, in any case, depend on the circumstances and the objectives of the modeller. One promising approach may be to use some combination of body size and habitat. Even the very complex West African fishery can be grouped into a limited number of species assemblages on the basis of habitat, specifically water depth and bottom type[4]. Within habitat divisions, grouping individuals by body size irrespective of taxonomy may not seem the most obvious thing to do, but since body size has rather predictable effects on an animal's vital rates[5], the approach has much to commend it. In the long term, successful predictions using aggregated models of complex multi-species fisheries could have profound implications for ecologists struggling to make sense of, and model, equally complex communities in other habitats.

Of course, even classical 'single-species' fisheries, such as cod or herring in the North Sea, or anchovies of Peru, are members of complex communities, providing both food and shelter and harbouring enemies and competitors[6]. A broader 'ecosystem' perspective on even a simple single-species fishery may suggest ways of using indicator species — sea-birds, for example — to monitor the health of fish stocks. There was disagreement among participants at the Dahlem Conference about the feasibility of such an approach, but it should not be dismissed out of hand. Certainly, reduced breeding success, or failure of sea-bird colonies to recover from a natural disaster that in the past has presented no problems, could be a clear indication that all is not well with the birds' food supply.

Another major theme of the meeting was the way unpredictable environmental events can lead to significant fluctuations in fish stocks, and thus to uncertainty in the catch from year to year, or over longer periods. In particular, it can be that the rates at which new recruits are produced are affected differently by some kinds of environmental fluctuations when the stock density is relatively low (as a result of heavy exploitation) than when it is relatively high (in its pristine state); this, in turn, may result in the yield becoming significantly more variable as fishing pressures increase. As observed at Dahlem[1,4] and before[7-9] such effects can lead to situations where it is better to fish less intensely, diminishing the fishing effort below that estimated to

* The Dahlem Conference on 'Exploitation of marine communities' was held in Berlin on 1–6 April 1984. The proceedings will be published as a book by Springer-Verlag, later this year.

give the maximum average yield in order substantially to reduce the severity of fluctuations in the yield. Such trading of risk against maximum possible return is more familiar in the management of university endowments.

In this general context, the conferees sought to identify some tentative patterns in the degree to which different kinds of fish stocks exhibit such variability. One suggestion was that species high in the food web tend to live longer, which in turn tends to make their population densities relatively less variable[10]. Another suggestion was that tropical species tend to live longer than ecologically similar temperate ones, again tending to make for relatively steadier dynamics[10], although for terrestrial insects Wolda and others have concluded that tropical populations do not fluctuate significantly less than temperate ones[11,12]. A more specific suggestion, tied to life-history details, is that species such as plaice or sole, which make their living on the essentially two-dimensional world of the sea floor, are thereby in a less chancy environment, and that this is why their stocks appear to fluctuate less than do many pelagic fish such as herring[13].

Troadec (Institut des Pêches Maritimes, Nantes) and others emphasized that fluctuations in the processing and marketing of fish are every bit as worrisome as fluctuations in the biological stock or in the catch. For fisheries that are relatively predictable, it may be desirable to bring industrial capacities for treatment, packaging and marketing fish products close to the minimum required to exploit the resource at some set average level, allowing for some fluctuations but not for gross overexpansion in good times and collapse in bad ones. This is easier said than done, but in the absence of such efforts at control then all factors — from biological harvesting to marketing — can create lags and inertia that end up greatly amplifying such fluctuations as are inherent in the system. Conversely, for fisheries that are intrinsically highly variable (either in yield or in species composition), as some pelagic fish seem to be, it may be best to aim for flexibility in harvesting, processing and marketing, given that 'cashing in' on the good years will have little repercussion on future biological events. Tidy though such a dichotomy is in principle, we really need to understand multispecies effects better before we can implement the ideas with any confidence[10].

As emphasized by Clark (University of British Columbia) and others in discussion, the real uncertainties in fish catches and marketing are further complicated by psychological aspects of uncertainty. Thus, for example, the macho acceptance of risks by some kind of fishermen can confound policies based on conservative rationality. Despite all the biological, economic, political and psychological problems that beset the management of both single-species and multispecies fisheries, the general tone of the final discussion was, we think, a cheerful one: our current understanding, though very far from complete, is probably enough to provide a basis for giving good advice for the management of most fisheries, at least in the short run. But in the long run, as stressed by Shepherd[14], in most cases we probably need kinds of information (knowledge of the absolute magnitude of stocks, and a full understanding of factors determining recruitment and mortality) that is not currently available. □

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Materials science

Superlattices, superdislocations and superalloys

from Robert W. Cahn

SUPERALLOYS, the key materials for making turbine blades and discs in jet engines, have been steadily improved since the introduction of the wartime 'Nimonics'; maximum service temperature has been progressively raised and engine efficiencies have been improved in parallel. But the available means for maintaining this rate of improvement are now more or less exhausted short of a radically new approach. Just such an approach is offered by some recent 'pure' research on one constituent phase of all superalloys, the nickel-based 'gamma prime' phase.

The diverse family of superalloys is, in essence, based on a single structural principle. They consist of a dispersion of particles of a coherent 'gamma prime' phase — Ni_3Al in its pure form but generally containing Ti and other elements — within a gamma phase, a matrix of nickel containing various solutes. Gamma and gamma prime are coherent in the sense that the lattices match up so closely that their interface is of very low energy; this in turn implies that the gamma prime particles are highly stable against growth. The other vital characteristic of these alloys is that the gamma prime phase is atomically ordered; in other words, it consists of a superlattice, like the much-studied Cu_3Au alloy, but unlike that alloy the order persists undiminished right up to the melting temperature. All sorts of other microstructural features have been introduced to add to the excellent creep resistance of superalloys at high temperatures, but the above features are the crucial ones.

Gamma prime has a remarkable property: whereas all ordinary alloys weaken as they are heated, the strength of an experimental alloy of pure Ni_3Al gamma prime phase increases as it is heated, almost until it melts. Plainly, this property is of central importance in determining the good creep resistance of superalloys as a class. Such anomalous mechanical behaviour as Ni_3Al and a number of other A_3B phases, such as Ni_3Ti. Much ink has been spilt in attempts to interpret this anomaly. It is known that dislocations move through highly ordered alloys in pairs, collectively termed 'superdislocations'. In a very stably ordered alloy these pairs of dislocations, which are joined by strips of disordered material called antiphase domain boundaries, are so close together that they can twist and turn and escape on to abnormal lattice planes. It is the twisting and turning that leads to the anomalous mechanical behaviour; at least, this is the current orthodoxy.

In superalloys cast in the normal way, the individual gamma prime particles — typically less than 1 μm across — each consist of a single domain. Consequently, it has not been possible to manipulate their domain size, which is an important variable in the general theory of the mechanical behaviour of ordered alloys. This situation has recently been changed, however, by research carried out in the Japanese laboratory at Tohoku University, where so much important metallurgical research has been done. An important paper from this group examines the behaviour of superalloys ultrarapidly quenched from the melt (Inoue, A., Tomioka, H. & Masumoto, T. *Met. Trans.* **14A**, 1367; 1983). At cooling rates approaching 10^6 K s^{-1}, there is not time for proper ordering and the phase consists of fine antiphase domains around 50 nm in size. The result, for a variety of phases of type Ni-Al-X, where X is Cr, Mo

or Fe, and the alloys consist entirely of gamma prime, is that both the strength and the ductility of the alloys are enhanced, whereas usually one of these improvements must be bought at the expense of the other. Once the quenched alloys are annealed, the domains grow (the state of order is improved) and the alloys become both weaker and less ductile. Unfortunately, Inoue *et al.* did not test the variation of the strength of the fine-domain structure with temperature; it is now important to know whether that is also subject to anomalous behaviour.

Another recent Japanese paper, from Tokyo (Ochiai, S., Oya, Y. & Suzuki, T. *Acta metall.* **32**, 289; 1984) reports the extent to which third metals can be dissolved in the gamma prime phases Ni_3Al, Ni_3Ga, Ni_3 and Ni_3Ge, and the superlattice site preferred by the third metal; these features are related to atomic size ratios and to the various bond strengths. In the present context, it is of particular interest that two of the authors of that paper previously studied the anomalous mechanical behaviour of a number of gamma prime phases (for example, Pt_3In and Pt_3Sn), finding, in general terms, that the more stable the ordered phase the more pronounced the anomalous strengthening with rise of temperature (Suzuki, T. & Oya, Y. *J. mater. Sci.* **16**, 2737; 1981). This seems consistent with the orthodox explanation of the anomaly, outlined above.

It should now be possible to answer some very interesting questions by rapid-quenching experiments on such 'model' gamma prime phases, both binary and ternary. First, does the size of the microdomains produced by quenching scale with the stability of the ordered phase? Second, how do the mechanical characteristics of these phases, particularly their anomalous behaviour, react to the introduction of imperfect order — that is, small domains? Finally, what are the consequences of the gradual healing of imperfect order by subsequent heat treatment? In this connection, it is probable that the domain size in a ternary alloy series based, say, on Ni_3Al or on Ni_3Ga, will be a function of concentration of the third element, and if so, it will be illuminating to see how the mechanical anomaly varies across a ternary series prepared by melt-quenching.

In this way, the curious mechanical characteristics of gamma prime can perhaps be related to the mechanical behaviour of imperfectly ordered phases such as Cu_3Au or FeCo, on which a large literature exists. In general, the strength of such alloys peaks at an intermediate degree of order, but the dependence of ductility on the degree of order has not been studied. The introduction of rapid quenching, it seems, may re-open the study of the mechanical characteristics of ordered phases, which has been in the doldrums of late. □

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Blood cell development

The message in the medium

from T.M. Dexter

EVERY minute of life, a human must produce an average of about 150 million red cells and 120 million granulocytes, which constitute the majority of white cells. Mature haematopoietic cells, such as these, are derived from several types of progenitor cell, each committed to develop into only one or two cell lineages; but all progenitor cells take their origin from the same 'stem' cell. The control of this developmental process — the regulation of 'production quotas' and of 'quality control' — is thought to reside in a family of haematopoietic growth factors. To a great extent, these growth factors are still ill-defined molecules present either in serum or in 'conditioned medium' — the fluid left after the growth of certain cells *in vitro*. But the techniques of molecular cloning are beginning to define the factors. One yielded to that approach earlier this year[1,2]; Gough *et al.*, from Melbourne, report their success with a second factor on page 763 of this issue[3]. The emerging data are bound to create widespread interest and excitement for developmental biologists.

The significance of these findings is best considered in the context of the overall process of the formation of blood cells which starts during embryogenesis with the laying down of a relatively small number of pluripotent haematopoietic stem cells. By virtue of their ability to self-renew, sufficient stem cells are generated not only to maintain their population throughout adult life but also to produce, by differentiation, the progenitors of all haematopoietic cells. It is obvious that the maintenance of haematopoiesis requires that a balance be maintained between self-renewal and differentiation. If too many stem cells were to differentiate, the system would soon undergo a decline; if too few differentiate, there will not be sufficient input into the progenitor cell compartment to meet the demand.

The progenitor cells, the immediate progeny of the stem cells, are recognized by their ability to undergo proliferation in soft-gel media *in vitro* to produce colonies of mature cells of the appropriate lineage. For many years it has been known that the survival, the proliferation and the development of these 'colony-forming' cells *in vitro* requires the continuous presence of specific growth regulatory molecules.

The story began with independent observations that colonies of granulocytes (neutrophils)[4] or macrophages[5] would develop in soft agar, provided that feeder cell layers or conditioned medium were present in the culture. Since then, modifications of this technique have provided the conditions in which the other three myeloid progenitor cell types — those that spawn red blood cells, platelets a eosinophils — can be stimulated to fo colonies (reviewed in ref.6). In all cas colony development requires the prese of ill-defined growth factors or 'colo stimulating factors'. Not surprising many workers have formulated (and still formulating) their own pet names these factors, causing much confusi both within and outside the field developmental haematology. Also, th has been a considerable variation in sources of the factors, the concentratic of factor used and the source of the resp ding cells (marrow, spleen, fetal live Because of this, it has been impossible determine, for example, whether the co mon progenitor of both granulocytes a macrophages responds to only one grow factor or to a family of growth facto Paradoxically, this initial lack of agre ment and standardization among t groups involved has been of some benef since several different and specific grow factors have been discovered as a result the variety of starting materials.

The use of conditioned media facilitate the growth of haematopoiet progenitor cells is still widespread. In th last five years, however, many of the acti growth factors have been purified to a parent homogeneity, enabling a clearer pi ture to emerge of their biological activiti and target cells. The properties and th nomenclature (still confusing) of some the growth factors for myeloid progenit cells are summarized in the table.

A simple interpretation of the data is th haematopoietic cell development regulated by a (limited) series of growt factors which are progressively restricted i their biological activities and target cell Thus, the recently cloned[1,2] growth facto IL-3 (for definition of abbreviations use see the table legend) is indifferent to lineag — promoting growth and development o all the different myeloid progenitor cell Moreover, it facilitates self-renewal o pluripotent (CFU-S) and multipoten (CFC-Mix) stem cells, and can 'immor talize' granulocyte precursors and mas cells *in vitro*. GM-CSF is more restricted stimulating both proliferation an development of only granulocyte macrophage and eosinophil colony forming cells and the proliferation, but no the subsequent development, of the CFC Mix and the red cell progenitor, BFU-E; t my knowledge, there is no evidence th GM-CSF stimulates self-renewal of any o these cell populations. The growth factor G-CSF and M-CSF are even more restricte in their activities.

Firm evidence that two of these growth factors are indeed quite distinct species

comes from the new data of Gough *et al.*[3], who have cloned and sequenced the cDNA of GM-CSF, and deduced the amino acid sequence of GM-CSF from the cDNA sequence. Also of significance is their observation that GM-CSF is encoded by only one gene; hence the reported differences in GM-CSF from different tissues probably reflect post-translational modifications. An intriguing additional finding by the Melbourne group is that, although they show no structural similarity, IL-3 can compete with GM-CSF for binding to its receptor (A.W. Burgess, personal communication). It seems, then, that some multipotential stem cells, progenitor cells and IL-3-dependent cell lines have both IL-3 and GM-CSF receptors; that IL-3 can bind to both receptors, but that GM-CSF can bind only to its own receptor. Since these two regulatory molecules appear to have quite distinct roles vis-a-vis self-renewal, proliferation and development, it may well be that the outcome of a response depends upon the relative concentrations of the two regulators and the number and accessibility of binding sites. Development may be further regulated by the lineage-restricted molecules G-CSF, M-CSF and erythropoietin.

The problems have by no means all been solved. Nonetheless, the way in which the haematopoietic cell growth factors interact to promote self-renewal, proliferation and development can now be studied in some detail *in vitro*. It will be just as important, of course, to determine whether such growth factors have a role *in vivo*. In the adult, haematopoiesis occurs in the marrow in association with a stromal cell meshwork. Evidence indicates that the stromal cells are not passive bystanders but themselves influence haematopoietic cell development, presumably by supplying the necessary cell matrix and diffusible molecules[7]. So far, it has been difficult to ascribe to IL-3 or GM-CSF a role in this process — the availability of cDNA probes should now help to resolve this problem.

The question of whether inappropriate expression of these growth factors has a role to play in leukaemogenesis can also now be addressed using the cloned cDNA. With this recent work, haematology has entered a new and exciting era. □

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Growth Factor	Other Names	Nature of Response	TARGET CELLS						
			CFU-S	CFC-Mix	BFU-E	GM-CFC (G / M)	Eos-CFC	Meg-CFC	Mast cells
IL-3	BPA, HCGF, Multi-CSF, MCGF, PCSF	Self-renewal	+	+	?	+ / - ?	ND	ND	+
		Proliferation	+	+	+	+ / +	+	+	+
		Development	ND	+	+	+ / +	+	+	+
GM-CSF	MGl-1GM CSF	Self-renewal	ND	-	-	- / -	-	ND	- ?
		Proliferation	ND	+	+	+ / +	+	-	- ?
		Development	ND	-	-	+ / +	+	-	+
G-CSF	MGl-2 D-Factor GM-DF	Self-renewal	ND	-	-	- / -	-	ND	-
		Proliferation	ND	+ ?	+ ?	+ / +	+ ?	ND	-
		Development	ND	-	-	+ / - ?	-	ND	-
M-CSF	CSF-1 L-cell CSF MGl-1M CSA	Self-renewal	-	-	-	- / -	-	-	-
		Proliferation	-	-	-	- ? / ++	-	-	-
		Development	-	-	-	- ? / ++	-	-	-

BIOLOGICAL ACTIVITIES AND TARGET CELLS OF MYELOID GROWTH FACTORS

The table is compiled from published data and is meant not to be a comprehensive survey of the field but to serve as a reference point for the various names and activities of the growth factors. + indicates a positive response, — a negative response, and ? an equivocal or controversial result. ND, not determined. The full names corresponding to the growth factor acronyms are as follows: IL-3, interleukin-3; BPA, burst-promoting activity; HCGF, haematopoietic cell growth factor; CSF, colony-stimulating factor; MCGF, mast cell growth factor; PCSF, P-cell-stimulating factor; GM-CSF, granulocyte/macrophage colony-stimulating factor; MGl-lGM, macrophage/granulocyte inducer—granulocyte macrophage; G-CSF, granulocyte colony-stimulating factor; MGl-2, macrophage/granulocyte inducer-2; D-factor, leukaemia cell-differentiation-inducing factor; GM-DF, granulocyte/macrophage and leukaemic cell differentiation-inducing factor; M-CSF, macrophage colony-stimulating factor; MGl-lM, macrophage/granulocyte inducer—macrophage specific; CSA, colony-stimulating activity; CSF-l, colony-stimulating factor-l. The target cells are: CFU-S, colony-forming unit—spleen, multipotential stem cells; CFC-Mix, colony-forming cell—mixed, a multipotential stem cell with the ability to form mixed myeloid colonies in soft agar; BFU-E, burst-forming unit—erythroid, a primitive erythroid progenitor cell assayed *in vitro*; GM-CFC, granulocyte/macrophage colony-forming cell which has an ability to form G (granulocytes), M (macrophages), or mixed colonies *in vitro*; Eos-CFC, eosinophil colony-forming cell; Meg-CFC, megakaryocyte colony-forming cell. BFU-E, GM-CFC, Eos-CFC and Meg-CFC form part of the so-called 'committed' progenitor-cell compartments.

Data analysis

The maximum entropy method

from John Skilling

MAXIMUM entropy is a remarkably powerful and general technique of data analysis. It can be used to sharpen up out-of-focus photographs (as in Fig. 1), to make maps of the radio sky, to generate images from medical scanners, to calculate spectra, to reconstruct electryn densities in a crystal and even to determine the positions of fuel rods in a nuclear reactor cooled with liquid sodium. It claims to be applicable whenever one needs to estimate a single vector of proportions $p = (p_1, p_2, \ldots, p_M)$ from seriously incomplete data, which could be fitted equally well by many different vectors. It claims to ignore what the proportions represent, which is why it can be applied to so many different inference problems. Maximum entropy always selects the simplest possible result, containing the bare minimum of structure needed to fit the data. Spurious detail is reduced as far as possible, which doubtless accounts for the practical success of the method.

The principle is always the same: one maximizes the entropy $S = -\Sigma_i p_i \log p_i$ subject to whatever constraints are imposed by the data. The theoretical basis of the method has often been less clear than the practical results however. Now papers by Shore and Johnson (*IEEE Trans. Inform. Theory* **IT-26,** 26; 1980 and **IT-29,** 942; 1983) and by Tikochinsky, Tishby and Levine (*Phys. Rev. Lett.* **52,** 1357; 1984) are making the justification for the maximum entropy method both clear and compelling.

Historically, the use of maximum entropy in data analysis has often been justified by supposing that the proportions were quantized, so that $p_i = n_i/N$. If N quanta were placed 'at random' into M available locations, the relative frequency of sets of occupation numbers n would be degeneracy $N!/\Pi n_i!$, which is equivalent to $\exp(NS)$, at least if N is large. Thus maximum entropy would be the same as maximum degeneracy, which could in turn be identified with maximum probability. Partly because of the dubious status of N and the quantization, this derivation has never been entirely convincing to scientists at large. An alternative justification has been to eschew quantization and identify S directly with (minus) the Shannon information content of p, considered as a vector of probabilities. One can then talk about minimizing the configurational information of p; but once again the rationale has not proved universally convincing.

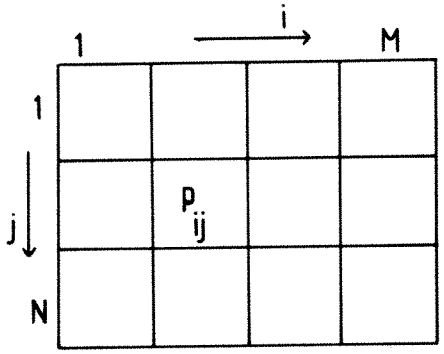


Fig. 2 For definition of i, j, M, N and p_{ij}, see text.

The new analyses reach a deeper level that bypasses the earlier derivations, and show that maximum entropy is the only consistent selection procedure. Let us start with Tikochinsky *et al.*, and with a diagram (Fig. 2), in which p_{ij} represents the probability of outcome i when performing experiment j. Tikochinsky *et al.* consider 'reproducible' experiments in which p_{ij} is independent of j, and in which incomplete data are acquired in the form of linear combinations $\langle A \rangle = \Sigma_i A_i p_i$. Because the data are incomplete (fewer than M combinations), the values of $\langle A \rangle$ do not fully determine the values of p. But if we must select just one single result, how should we go about it? Suppose the experiment is repeated N times. We are at liberty to treat each experiment independently. In this case, our selection algorithm (whatever it might be) will choose the same proportions p_i for each experiment j, and these could be combined to give an overall distribution P_i for the outcomes i after the N repetitions. We are also at liberty to treat the whole as one large experiment, in which case our selection algorithm will immediately produce an overall distribution Q_i. Tikochinsky *et al.* point out that we must surely require $P_i \equiv Q_i$ if data from reproducible experiments are to be combined consistently.

Repeating or averaging an experiment does not change its outcome, and it should not change the selected result either. In ten short equations, Tikochinsky *et al.* proceed to prove that the consistency requirements is satisfied if, and only if, the selection algorithm is maximum entropy. In a brilliantly simple way, entropy emerges as an elementary consequence of consistent reasoning.

The consistency approach can be taken even further. Tikochinsky *et al.* couch their analysis in terms of probability distributions, but it often seems a little artificial to have to identify a set of physical proportions with a set of probabilities of outcomes of a random experiment. Proportions and probabilities are, of course, isomorphic, but why should we need to introduce the idea of randomness at all? And why should we need to restrict ourselves to linear data? The answer to both questions is that we need not.

Let us return to Fig. 2, but let p_{ij} now represent the proportion of the total observed quantity assigned to cell i, j of the $M \times N$ array ($\Sigma_{ij} p_{ij} = 1$): we could be considering a two-dimensional digitized picture. Suppose that the data are some arbitrary functions $U(u)$ and $V(v)$ of the marginals $u_i = \Sigma_j p_{ij}$ and $v_j = \Sigma_i p_{ij}$. We are at liberty to ignore the 'north–south' v-structure, and use our selection algorithm to choose an 'east–west' distribution u_i which fits the data U. Similarly, we can select a north–south distribution v_j which fits the data V, without reference to U. Finally, we are at liberty to use all the data, U and V together, to produce immediately an overall distribution p_{ij}. We must surely require $p_{ij} = u_i v_j$ if data from independent experiments are to be combined consistently. North–south data must not interfere with east–west structure, and vice versa. This will be the case if, and only if, the selection algorithm is maximum entropy. Again, entropy emerges as an elementary consequence of consistent reasoning.

This is the derivation of entropy which we at Cambridge now prefer (see our rigorously informal presentation in *Indirect Imaging* ed. Roberts, J.A., Cambridge University Press; 1984, a more mathematical account of which has been



Fig. 1 What maximum entropy techniques can do for car-spotters, including the police.

submitted to *Acta crystallogr.* A). We obtained our derivation from Shore and Johnson's papers, in which the notion of independent dimensions is refined to a requirement of system independence, and the whole treatment is given a proper axiomatic foundation. Shore and Johnson give four axioms — uniqueness, invariance, system independence and subset independence — which must be satisfied by any consistent selection algorithm. In careful and formal work that is remarkably similar to an axiomatic derivation of Shannon information, Shore and Johnson derive the entropy formula, generalized to $S = -\Sigma_i p_i \log(p_i/m_i)$ to allow for a possible prior model m. Although they describe p as a probability distribution, this is unnecessary; their work applies equally well to any distribution of proportions, and the technical words 'probability' and 'information' need never appear.

These ideas justify the fundamental claims made for maximum entropy in data analysis, and it is clear that we need not quantify our preference for the maximum entropy selection by anything other than the numerical value of S. It is sufficient to know that we must use maximum entropy — or lay ourselves open to the charge of inconsistency. Let's get on with it. □

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Palaeobotany

Early evolution of leaves

from J.B. Richardson

THE origin and evolution of leaves was a major event in land plant evolution and must have affected all other life on land. Much remains to be learnt, however, of when they first appeared and how they evolved. Fossils described on page 785 of this issue by Gensel[1] confirm what had already been suspected from compression fossils from the Gaspé Peninsula: by the end of the Lower Devonian, vascular plant evolution was quite advanced with complex forms present. The presence of divided non-laminate leaves at the end of the Lower Devonian is already documented[2] but Gensel[1] describes changes in anatomy in a lateral branch system suggestive of the early evolution of a megaphyll — a laminated leaf with a complex pattern of venation.

Did leaves arise as Zimmermann suggests in his telome theory[3], through evolutionary overtopping, planation and webbing? A telome is the most distal dichotomy of a plant. Overtopping describes what occurs if one branch of the dichotomy develops more than the other. The overtopped branches would diminish in size, eventually tranforming the equal dichotomies into a main stem with lateral branches. Planation describes the change from a three-dimensional arrangement into a single plane of neighbouring telomes. Webbing, the joining of tissues between the lateral branches of the planar telomes, would result in the familiar lamina (leaf-like structure) with dichotomous venation.

This is what Zimmerman proposed but how is the theory testable? One of the restraints on evolutionary hypothesis is the geological record. Do these morphological features appear in the geological succession in the order demanded by the theory? The answer is both yes and no. Simple dichotomously-branched axes have been recorded in the mid-Silurian (Wenlock)[4], while by the late Silurian, rhyniopsids, with a similar appearance, were common but not diverse. Overtopping was present in the early Devonian (Lower Gedinnian)[5]. At that time there was considerable diversity of the rhyniopsids, and zosterophylls, distinguishable from the rhyniopsids on the basis of lateral sporangia, were present[6]. Later, during the Siegenian, the zosterophylls diversified[7] and the trimerophytes, such as *Dawsonites*, appeared. Later still, in the Lower Emsian of Belgium, a similar flora along with several *Psilophyton* spp. was present[8] and work by Andrews indicates a high diversity of plants at that time (see *inter alia* ref.9). Trimerophytes in the Emsian exemplify overtopping and reduction of laterals; as Stewart[10] states "Trimerophytopsida exhibits almost every branching pattern to be found in megaphyllous vascular plants". Thus, as re-emphasized by Gensel's paper, Zimmermann's leaf-forming processes, apart from planation and possibly webbing, had happened by the late Lower Devonian and much of the geological record supports the telome theory on the origin of megaphylls.

Zimmermann derived a great variety of leaves and leaf-like structures, including microphyllous 'leaves' of the lycopsida, from a rhyniopsid-like ancestor. Bower's enation theory[11] is an alternative proposal for deriving microphylls — small leaves with a single central vein. In the telome theory, the microphylls arise by reduction of bunches (trusses) of telomes. Bower's theory is that bumps (enations) emerged from the surface of the stems. As these enations extended (in the evolutionary sense) they developed vascular tissue, first at the base of the enation (as in *Asteroxylon*) and then throughout the enation, as in *Baragwanathia*, which thus became a true microphyll. According to the telome theory this series would be reversed: reduced vascularized microphylls would precede unvascularized outgrowths. Which



100 years ago

SOME forty years ago Dr. Joule raised the question whether a body that is magnetised undergoes any changes in its temperature; but the question has not yet received a definite solution, the rise of temperature which accompanies magnetisation being ascribed by some to induction currents, and not directly to magnetism. While recognising the influence of the former, Mr. Borgman has tried to show that there is also a change of temperature due to the magnetism and demagnetisation, and that the amount of heat thus disengaged is proportionate to the squares of the temporary magnetism. M. Bachmetieff, having made, at the University of Zurich, an extensive series of experiments, the first part of which is now published in the *Journal of the Russian Chemical Society* (vol. xvi. fasc. 3), arrives at the conclusion that magnetism, *by itself*, produces variations of temperature in magnetised bodies, and that this "magnetic heat" is equal to the product of the magnetic moment by the magnetising force multiplied by a constant; it increases also, within a certain limit, with the frequency of the interruptions of the magnetising current, and increases still more when the direction of the current is alternately changed. Its amount is not equal throughout the length of an iron cylinder, reaching its maximum about its middle and decreasing towards its ends. Its cause must be searched for in purely mechanical forces.

RATHER a strange occurence came recently before my notice, and thinking perhaps you might care to insert it in your columns, I send you the facts of the circumstance. A few days since, towards evening, I killed a snake just close behind my house; it measured about a yard and a half in length, was one of the most deadly of the numerous kinds of snakes found in Java, and bears the name of "Oelar belang." On examining it later I found what I thought to be the tail of another small snake protruding from its mouth, but on pulling it out I was greatly surprised to discover that it was really a snake of the same species, and of almost the same length. There was certainly not more than three inches' difference in the length of the two snakes, and at the time I killed the outside snake only about an inch and a half or two inches of the tail of the one he had swallowed protruded from his mouth. The natives here say that the two snakes must have been fighting, the victor afterwards swallowing his opponent. I should be pleased to know whether such an instance has ever before been brought before your notice, or whether it is really an uncommon case.
Soemedang, Java.

M. MONTGIGNY has recently published a pamphlet on the influence of the atmosphere in the apparition of colours seen in the scintillation of stars. He has previously noticed that there is a great predominance of blue in the scintillating colour when rain is approaching, and he is now so convinced of the accuracy of this forecast that it is included among others in the *Bulletin Météorologique* published by the Observatory of Brussels.

From *Nature* **30**, 3 July 1884.

theory is correct may seem simple to resolve by accurate age determination of plants whose anatomy is well known. But Stewart[10] proposed at least two lines of lycopsid evolution in the Lower Devonian, one evolving by the enation 'process' and the other according to the telome theory. Earlier, Schweitzer[12] suggested several lines. A great deal of controversy revolves around plants with 'microphylls'. In the Northern Hemisphere the earliest 'microphylls' are on the Gedinnian *Drepanophycus*[13] and 'microphylls' (with no trace of a vein) are known in *Asteroxylon*. Unfortunately many of the main morphological features of these plants, such as sporangia and spores, are unknown or poorly described. In the Southern Hemisphere much controversy surrounds *Baragwanathia* which occurs in Australia in rocks recently re-dated as Upper Silurian (Ludlow). If *Baragwanathia* is now correctly dated and is a true lycopsid then its presence in the Silurian may cast doubts on the origin of the lycopsids from the rhyniophytes.

With the expectation of continued discoveries by those who have already contributed to our knowledge of the early land floras and careful documentation of the geological age of their finds, the next few years promise to be as exciting as, but probably no less controversial than, the recent past. □

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Immunology

The T-cell receptor completed

from Guil Winchester

T LYMPHOCYTES occupy a central position in the immune response. Like B lymphocytes they are capable of responding to an enormous variety of foreign antigens but they also control and coordinate the activity of other cells in the immune system. It was therefore a source of frustration and embarrassment to immunologists that the surface structure by which T cells recognize antigen remained obstinately invisible for many years. Over the past eighteen months, however, a number of consistent results have gradually been building up into a picture of the T-cell receptor. The publication of the complete sequence of the cDNAs for both subunits of a T-cell receptor on page 757 of this issue[1] completes the task.

The first glimpse of the receptor came last year when monoclonal antibodies, specific for individual T-cell clones, were found to precipitate protein dimers that seemed to be essential for the activation of T cells by antigens[2,3]. The polypeptide subunits of the dimers have slightly different molecular weights; each polypeptide contains constant regions, which are common to several clones, and variable regions which are clone-specific[4-6]. The protein chemists were then overtaken by the molecular biologists. In March of this year, two groups simultaneously published the sequence of cDNA clones from both mouse[7,8] and man[9] which now seem certain to code for the β-subunit of the receptor (Reinherz cited in ref.1). Since then, a genomic sequence for the β-subunit from mouse has been published[10]. The data show that, like the immunoglobulin gene, the β-subunit gene is split into segments in the germ line and the segments are brought together in differentiated cells to form a functional gene. Since each segment is present in a number of forms, which can be randomly assorted, the genes can encode an enormous number of receptors.

The α-subunit has, however, remained obscure. Now Susumu Tonegawa and his colleagues have isolated and sequenced two distinct cDNA clones from mouse, both of which are expressed and rearranged specifically in T cells[1]. Their sequence identifies one as the β-subunit and the other has all the properties predicted for the α-subunit.

The overall structure of the receptor presented by Saito *et al.* looks remarkably like two immunoglobulin light chains, both anchored in the membrane by a stretch of hydrophobic amino acids. Each chain has two domains on the external surface of the membrane, one constant and one variable, with the variable region at the amino-terminal end of the molecule. Between the constant domain and the cell membrane there is a short sequence which contains the cysteine that is thought to bind the two subunits together; this is followed by a sequence that spans the membrane and a short tail found in the cytoplasm. The α-subunit (which is the larger) has a slightly longer tail which contains an extra cysteine. Saito *et al.* suggest that this cysteine may form 'dynamic interactions' with other molecules. One candidate would be the T3 protein which is found in all mature human T cells and which seems to be involved in the triggering of T cells by antigen (for a review see ref.11). Removal of T3 from the surface (induced by anti-T3 antibodies) is accompanied by loss of the T-cell antigen receptor, implying that they are physically associated.

The new data provide Saito *et al.* with the first opportunity to compare receptor molecules from different T-cell subsets. The earlier clones encoding the β-subunit were isolated from T-helper cells, which activate the various effector cells in the immune system, while the cDNA clones described by Saito *et al.* come from cytotoxic T cells whose primary role is the direct killing of virally infected cells.

Although only the β-subunits from different subsets can be compared, the authors draw two tentative conclusions. One is that the variable-region genes used by cytotoxic and helper cells may well come from different gene pools; the two variable-region genes compared so far are only 20 per cent similar, which is comparable to the degree of similarity between the constant-region genes of the β-subunit of the T-cell receptor and those of the immunoglobulin light chains. Since helper-T cells usually recognize antigen in association with class II major histocompatibility complex (MHC) products, while cytotoxic cells recognize antigen in association with class I MHC products, dissimilarity between the variable regions of helper cells and cytotoxic cells is not unexpected. Moreover, it is compatible with the observation that helper cells and cytotoxic cells have different antigen repertoires.

More surprising is the fact that the β-subunit gene from cytotoxic cells shares a constant-region sequence with one of the helper-cell genes, the identity extending over the entire 3′-untranslated region. Differences between the constant regions of different T-cell subsets might have been expected; in an immunoglobulin molecule the constant region of the heavy chain determines its functional class. Two possibilities that might account for this anomaly are discussed by Saito *et al.* One is simply that functional differences between the two cell types lie on the constant region of the α-chain. The other is that the T-cell receptor is purely a recognition molecule and has a non-specific role in triggering effector functions. This question should be answered as soon as the α-subunit from a T-helper cell has been sequenced. At the rate events are moving in the field that should not be long delayed. □

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Astronomy

Humps and superhumps

from Andrew King

CATACLYSMIC variables are close binary systems with orbital periods of a few hours, in which a white dwarf accretes matter from a faint low-mass companion star. They have shown themselves to be ideal systems for studying accretion processes, allowing frequent and stringent tests of theoretical ideas. An important new test is reported by Whitehurst, Bath and Charles on page 768 of this issue.

Cataclysmics show convincing evidence of possessing accretion discs; the accreting matter spirals slowly in to the white dwarf through a sequence of Kepler orbits (centrifugal force balancing gravity) under the action of dissipative forces, gradually giving up its gravitational potential energy to radiation. The stability of such a disc is a matter of great importance and under active debate, with dwarf novae providing the test ground. These are a class of cataclysmics which at irregular intervals (weeks to months) brighten dramatically (factors of about 10) in a day or less, remaining at this 'outburst' level for anything up to about 10 days (occasionally longer) before decaying back to their 'quiescent' level in 1 to 3 days. Two types of model have been proposed to explain this behaviour. In one, put forward by G.T. Bath and co-workers (University of Oxford), the 'donor' star (or secondary) is slightly unstable, and at intervals feeds bursts of mass transfer into the accretion disc. In the other, the outburst is a consequence of instabilities purely within the disc, without any change in the state of the secondary: work on these lines is being pursued by groups in Munich, Texas and Santa Cruz (see *Nature* **302**, 12; 1983). In their paper on page 768, Whitehurst *et al.* attempt to broaden the debate to include the phenomenon of 'superhumps'.

Superhumps are periodic increases in brightness (of up to 40 per cent) that are observed during the occasional 'superoutbursts', lasting about 12 days, that are additional to the 'normal' outbursts of one subclass of dwarf novae (the SU Ursae Majoris systems). Fascinatingly, the superhumps repeat on a period, P', that is a few per cent longer than the orbital period, and so cannot be due to some feature locked to the orbital rotation, such as an eclipse, for example. (It is an intriguing question whether the SU Ursae Majoris phenomenon is restricted to short-period dwarf novae, or whether observational selection effects prevent us recognizing it in longer-period systems. See Warner, B. in *Interacting Binaries*, eds Pringle, J.E.P. & Wade, R., Cambridge University Press; in the press, and van Paradijs, J. *Astr. Astrophys.* **125**, L16; 1983, for a discussion).

Whitehurst *et al.* focus on two systems which have orbital inclinations high enough that the secondary star periodically crosses our line of sight to the accretion disc, causing a deep eclipse, repeating at the orbital period, P. Painstaking efforts by B. Warner (Cape Town University) and W. Krzeminski (now Las Campanas) have yielded optical light curves through superoutbursts of these systems. The deep orbital eclipses are still there, indicating that most of the extra superoutburst light comes from the accretion disc. The interesting question which Whitehurst *et al.* address is whether the superhump light is eclipsed. Since the superhumps 'march' slowly through the orbital light curve on the beat period $(1/P - 1/P')^{-1}$ of about 2–3 days, there are phases when the orbital eclipse falls right on top of the superhump. If the superhump light were totally eclipsed at these phases, the residual light at the bottom of the eclipse should be exactly the same as that at phases where eclipse and superhump do not coincide. If, on the contrary, the superhump light were entirely uneclipsed, this residual light should vary in the same manner as the superhump itself. Using data from several eclipses, Whitehurst *et al.* conclude that the latter is the case. This virtually forces the site of the superhump phenomenon to be on the secondary star, as only a rather contrived model could restrict it to the small part of the accretion disc which is never eclipsed.

Activity on the secondary star is, of course, exactly what is invoked by the Bath model for dwarf nova outbursts; in the disc instability picture such activity would require a separate explanation. Moreover, most models place the superhump site on the accretion disc, where it would be partially eclipsed (see Warner, *op. cit.*). Whitehurst *et al.* suggest that the superhump is due to a bright spot on the secondary where part of the stellar envelope is lost to the white dwarf to cause the superoutburst: the spot is larger than usual since superoutbursts last longer than normal outbursts. The superhump period P' then represents the rotation period of the secondary; that is indeed expected to be just slightly longer than the orbital period P because of the synchronizing effect of tidal torques in a close binary.

All this presupposes that one accepts Whitehurst *et al.*'s conclusion that the superhumps are entirely uneclipsed. Because the superhump does not repeat precisely on each appearance, an escape hatch from this conclusion is still ajar, in that partially eclipsed superhumps may be allowable (see Whitehurst *et al.*'s Fig. 2). Whether they occur is a question that can be settled observationally, and should soon be, with the powerful stimulus of the new paper. But even when this question is disposed of, others will remain, such as why the superhump radiation pattern has such a highly structured and repeating nature. Superhumps and superoutbursts look set to challenge theoreticians and observers for some time to come. □

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Molecular biology

Transposable elements and suppressor genes

from Ian J. Jackson

MOLECULAR biologists like mutations. Determination of the molecular basis of genetic mutations has already revealed unsuspected means by which gene expression can be disrupted. Now a class of mutant genes in species as diverse as yeast, *Drosophila* and mouse is providing more surprises as to mechanisms of mutagenesis, and may well yield information about the regulation of normal development. The mutations are caused by the insertion of transposable genetic elements in or near a gene and are suppressed by recessive genes which map elsewhere in the genome. Recently published work together with data discussed at a meeting in Colorado* reveals a remarkably unified theme among the mutations at the structural level, although the modes of action of the suppressor genes are proving to be somewhat enigmatic.

The transposable elements that cause the mutations include four classes: Ty of yeast, *copia* and *gypsy* of *Drosophila* and the ecotropic endogenous retroviruses of mouse. Each consists of a few kilobases of DNA flanked at either end by a duplicated sequence of several hundred base pairs, the long terminal repeat (LTR). In all cases where they cause a suppressible mutation, the transposable elements are inserted not into coding DNA but into an intervening or flanking sequence. After insertion, homologous recombination between the two LTRs can result in excision of the internal sequences, leaving a single LTR behind, and often resulting in total or partial reversion of the mutation. In all three species this occurs at a frequency of about 10^{-5}–10^{-6} per generation. Suppression of these

* UCLA Symposium, 'Genome rearrangement', held at Steamboat Springs, Colorado, 8–12 April 1984.

mutations, however, is not caused by an increase in the excision rate of the element. Instead, in some cases at least, it seems that the mutant phenotype is a result of transcription of the inserted element, and it is on this transcription that the suppressor genes act. All four classes of element have a promoter of transcription in one LTR and a terminator in the other, and thus will produce, under the right circumstances and where there has been no internal excision, a RNA molecule running the full length of the element. Whether, in all cases, this transcription causes the mutant phenotype remains to be seen, but the nature of the suppression and the wild-type function of the suppressor genes should prove revealing.

One good example of the phenomenon is the production of the auxotrophic mutations *his4-912* and *his4-917* in yeast by insertion of a Ty element upstream of the *HIS4* gene[1]. The *spt3-* mutation (formerly *spm3-*) of yeast suppresses *his4-917*, in which transcription of the Ty is away from the *HIS4* gene, but it does not suppress *his4-912*, in which transcription is towards *HIS4*. However, excision of most of Ty leaving behind a solo δ-sequence (the LTR of Ty) results in a cold-sensitive mutant phenotype which is then suppressible by *spt3-*. This arrangement has allowed Gerry Fink (Whitehead Institute/MIT) to examine the expression of the gene. At low temperatures, transcription of the *HIS4* gene initiates from the δ-sequence, 5′ of the gene, instead of the correct site. The resultant RNA is inactive. In the presence of *spt3-*, transcription starts from the correct position at all temperatures and all transcription from the normal origin in all δ-sequences of the cell is inhibited. Thus the simplest explanation for the mechanism of suppression is that transcription from the δ-sequences of the Ty elements placed in critical regions 5′ of a gene will prevent normal gene expression, and that *spt3-* mutants lack the ability to transcribe some or all of the Tys, so relieving the mutation. In this model the wild-type *SPT3* gene encodes a transcription factor required for initiation of transcription from δ-sequences. Perhaps the Ty mutations that are not suppressed by the *spt3-* mutation are transcribed by a different mode.

Inevitably, the genetics of the *Drosophila* suppressors is much more complex and the molecular biology less clearcut. The *suppressor of hairy wing* gene, *su(Hw)*, not only suppresses *hairy wing*, but a number of alleles at other loci, including *cut*, *scute*, *lozenge*, *forked*, *rudimentary* and several alleles of the homoeotic complex, *bithorax*. In work published last year, Modolell, Bender and Meselson reported the cloning of DNA from five suppressible alleles of *bithorax*[2]. Each of them contained the same transposable element, *gypsy*; indeed, all the genes affected by *su(Hw)*, except *rudimentary*, contained *gypsy* elements. Modolell *et al.* suggested that the product of the wild-type *su(Hw)* gene somehow interacts with *gypsy* to cause the mutations; a mutation at *su(Hw)* prevents this interaction and so suppresses the mutagenic effect of *gypsy*. At the Colorado meeting, Welcome Bender (Harvard University) presented data from *bithorax* which indicate that it may be transcription of the *gypsy* element which is causing the mutant phenotype. It cannot simply be the effect of insertion of *gypsy* into *bithorax* because other insertions at the same locations have a less pronounced effect. Similarly, chromosomal breaks have a different effect from *gypsy* insertion, suggesting that premature termination in the element is not to blame. Bender has gone on to show that in the presence of homozygous *su(Hw)*, transcription of the total population of *gypsy* elements is reduced fivefold. Whether the reduction of transcription is more from certain elements than from others is not known, but an effect on gene expression via transcription of the element is certainly strongly suggested. The problem remains of why *su(Hw)* suppresses two alleles of *rudimentary* which do not contain *gypsy* sequences.

The precise mode of action of the *su(Hw)* suppressor gene and the function of the product of its wild type have not emerged, but the observations of Paul Bingham (SUNY, Stonybrook) in relation to the *white-apricot* gene of *Drosophila* may be enlightening. The *white-apricot* (w^a) phenotype is caused by insertion of the transposable element, *copia*, into an intervening sequence of the *white* eye-colour gene (refs 3, 4 and K. O'Hare, personal communication). The effect of the w^a gene is suppressed by *suppressor of white-apricot*, *su*(w^a), which results in a darker eye colour, but can also be enhanced to produce a paler eye-colour by *suppressor of forked*, *su(f)*. Somewhat enigmatically, *su(f)* also suppresses the same alleles of *forked* and *lozenge* that are suppressed by *su(Hw)* and contain *gypsy* insertions. Bingham reported that a partial revertant of w^a, containing a single LTR insertion in place of the whole *copia*, was not affected by either *su*(w^a) or *su(f)*. This again suggests some effect of transcription of the element on the gene into which it has been inserted. This is further supported by evidence that the very low level of normal *white* transcript produced from the w^a gene was reduced still further in the presence of *su(f)*, but increased several fold when *su*(w^a) was present (Gerry Rubin, University of California, Berkeley).

To investigate the nature of suppression and the normal function of the suppressor genes further, Bingham has made several new mutations at *su*(w^a), almost all of which are recessive lethals. The exception, when homozygous, produces a fly that lacks wings and halteres, in a manner which suggests that specific cells of the imaginal discs die or fail to develop. The normal function of *su*(w^a), suggests Bingham, is to provide developmental signals within *Drosophila*. How the *copia* element in the w^a gene responds to these signals in such a way as to restore a normal white phenotype is a mystery. A guess would be that in certain cells the element is dependent for transcription upon these developmental signals, and that some mutations of the signal prevent the expression of this particular *copia*, and thus suppress w^a, whilst still allowing normal development of the organism.

Hope Sweet, of the Jackson Laboratories, reported last year the identification of the first recessive suppressor mutation in the mouse[5]. The gene, *dilute suppressor* (*dsu*), suppresses the *dilute* mutation (*d*) *Dilute* produces a pale coat colour. Jenkins *et al.* have shown it to be due to the insertion of an endogenous retrovirus into the mouse genome[6,7]. Revertants that are left with only one of the two LTRs have normal coat colour. Jenkins (University of Cincinnati) reported that the retrovirus seems to be inserted into a noncoding segment of DNA, so the means by which the retrovirus disrupts expression of the *dilute* gene are not evident. Perhaps the presence of the intact viral DNA prevents initiation, or causes premature termination or incorrect splicing of the *dilute* transcript. Although mice bearing the retrovirus do not become viraemic, the viral promoter might be active in melanocytes in a way that prevents correct *dilute* expression and, so, coat colour.

The effect of *dsu* on other genes may help explain how it suppresses *d* and, in turn, the cause of the *d* mutation. An allele of *d*, *dilute-lethal* (d^l), causes both a pale coat and a fatal neurological disorder. The coat coloration effect but not the lethality of d^l is suppressed by *dsu*. Hope Sweet (personal communication) has also shown that *dsu* suppresses *ashen*, a mutation which mimics the phenotype of *dilute*. Neither d^l nor *ashen* is associated with a retrovirus of the same type that causes *dilute*, although the presence of another virus or solo LTR has not been excluded. It seems, however, that the action of *dsu* is involved more directly in affecting the melanocyte morphology which gives rise to the dilute or ashen phenotype than in the expression of the genes themselves. How a recessive suppressor could act in this way, and the function of the wild-type *dsu*, are of considerable interest; experiments in progress at Cincinatti and the Jackson Laboratories should illuminate these points. □

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A large-scale isotope anomaly in the Southern Hemisphere mantle

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Basalts from many Southern Hemisphere regions have anomalous Sr and Pb isotopic characteristics. This article shows that the isotopic mantle anomaly is globe-encircling in extent, centred on latitude 30° S. Arguments suggesting that this mantle anomaly has been in existence for billions of years place severe constraints on mantle convection models.

RECENTLY, Dupré and Allègre[1] made an important observation concerning the existence of a large domain (10^8 km^2), centred in the Indian Ocean, which is delineated by oceanic island and oceanic crust basalts having anomalous Sr, Nd and Pb isotopic signatures. The existing isotopic data for young basalts show that this isotopic mantle domain, here termed the Dupal[1] anomaly, can be traced nearly continuously around the Southern Hemisphere between the Equator and 60° S. Part of this anomaly was previously delineated in the central Pacific[2]. This is the largest mantle geochemical domain yet delineated; I argue that this isotope domain was produced early in Earth history by core–mantle–crust differentiation processes. Survival of this anomaly strongly constrains models of mantle convection.

Isotope criteria

Three criteria are used to quantify the Dupal anomaly: (1) $^{207}Pb/^{204}Pb$ and (2) $^{208}Pb/^{204}Pb$ deviation from a Northern Hemisphere reference line (NHRL), (3) absolute value of $^{87}Sr/^{86}Sr$. Choice of a Northern Hemisphere Pb reference line is arbitrary, but convenient because of the abundance of data and essential colinearity of mid-oceanic ridge basalt (MORB) and oceanic island basalt (OIB) data on Pb–Pb plots. The reference lines were drawn to include MORB (both N-type and E-type), the islands of Hawaii, Iceland, Azores, Canaries and Cape Verde[3], and the north-east Pacific seamounts[4]. The equations of the adopted reference lines (NHRL) are:

$$^{207}Pb/^{204}Pb = 0.1084(^{206}Pb/^{204}Pb) + 13.491$$

$$^{208}Pb/^{204}Pb = 1.209(^{206}Pb/^{204}Pb) + 15.627$$

The magnitude of the isotopic anomaly of any given data set (DS) is then expressed as the vertical deviation in $^{207}Pb/^{204}Pb$ or $^{208}Pb/^{204}Pb$ from these reference lines:

$$\Delta 7/4 = [(^{207}Pb/^{204}Pb)_{DS} - (^{207}Pb/^{204}Pb)_{NHRL}]100$$

$$\Delta 8/4 = [(^{208}Pb/^{204}Pb)_{DS} - (^{208}Pb/^{204}Pb)_{NHRL}]100$$

For $^{87}Sr/^{86}Sr$, I use the absolute magnitude of the ratio, expressed as:

$$\Delta Sr = [(^{87}Sr/^{86}Sr)_{DS} - 0.7]10^4$$

The coherence between these three anomaly criteria is fairly good. Figure 1 shows the correlation between $\Delta 7/4$, $\Delta 8/4$ and ΔSr. The limiting precision of these criteria is that derived from the isotopic measurements themselves, and is largest for $\Delta 7/4$ because mass spectrometer fractionation effects are large relative to the total variation of $\Delta 7/4$ (measurement precision of ±2 units is 10% of total $\Delta 7/4$ variation of 20 units) and the fractionation vector is steeply inclined to the reference line. The $\Delta 8/4$ criterion is more robust because the fractionation vector is semi-parallel to the reference line and the total spread in $\Delta 8/4$ is large (measurement precision of ±5 units is only 3% of the total $\Delta 8/4$ variation of 190 units). Likewise, the measurement precision of ΔSr is small with respect to the observed spread. There are, of course, significant and unconstrained sources of uncertainty due to 'geological scatter'—the fact that averaged island data sets are used which may show large isotopic variations internally. For data sets involving many samples (Iceland, Hawaii, Galapagos), this scatter may be effectively averaged out; for some data sets, however, only one or two analyses are available and the problem of representativeness may be very serious. Note, however, that the variations of Pb isotopic ratios within a given island basalt suite are commonly sub-parallel to the reference lines, so that the calculated Δ values are insensitive to this kind of intra-island isotopic heterogeneity.

Data

The basic data set used is that compiled by Zindler *et al.*[3]; these data have been augmented with data from other studies, including some basalts from continental environments. The basic criterion has been to restrict the data sets to basaltic rocks and, for the continental environments, I have used only basalts for which Pb data exists, and which carry ultramafic mantle xenoliths (as a precaution against crustal contamination). These continental basalts are included because they seem to fit the picture, and fill in several important geographical gaps. Several of the basalt suites may be related to subduction zones (Pribilof's and Fiji); in the case of Fiji, only the young alkali basalt suite was used, as this is claimed to postdate subduction involvement[4]. Several suites range up to 85 Myr in age (Walvis Ridge, Rio Grande Rise, Ninety-East Ridge, New England seamounts); while the age correction will not significantly affect the anomaly criterion, it does affect the geographical position of these suites. (When formed, the Ninety-East Ridge samples were located some 40°–50° south of their present position.) Inclusion of these older suites shows that the Dupal anomaly has been a persistent feature of the mantle for at least 10^8 yr. Table 1 summarizes the data suites used, not including the Pacific ocean Sr data of Hedge[2] and Duncan and Compston[5].

World maps of these data, contoured for the three anomaly criteria, are shown in Fig. 2. While the configuration of the Dupal anomaly varies between the three different anomaly criteria, it is, nevertheless, a striking feature on all three maps, occupying a band of some 60° of latitude centred on 30°–40° S. The strongest maximum in the anomaly for all three criteria stretches from the southern mid-Atlantic Ridge to the central Indian ocean; a second maximum shows up on the ΔSr and $\Delta 8/4$ plots in the central Pacific. Very few Pb data have yet been published for this region. Based on available isotopic data (both Pb and Sr)[2,5–9], it appears that a configuration similar to the steep gradient between the islands of Tristan, Gough and St Helena in the Atlantic may exist, with Samoa, Raratonga and the Marquesas Islands reflecting a Dupal signature and the Tubuai Islands and southern Tuamoto Islands showing low anomaly values, similar to St Helena. Note that these two regions are almost antipodal to each other, with a near-equatorial two-fold axis of symmetry located at ~100° E.

Aside from the central Pacific, the other areas where there is little data coverage are the high latitudes; these regions are

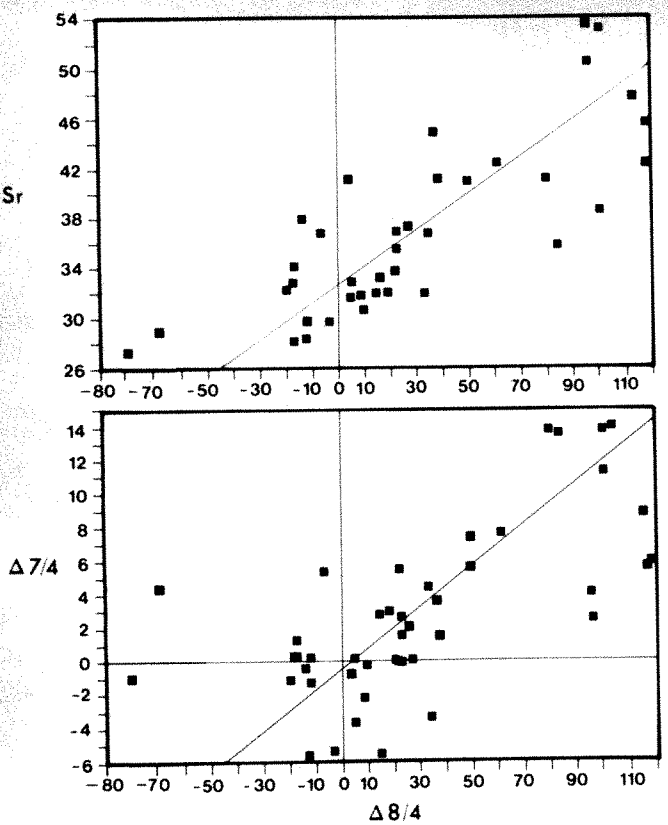


Fig. 1 Anomaly criteria ΔSr and Δ7/4 plotted against Δ8/4; data from Table 1. See text for definition of anomaly criteria. Best fit regression lines are: $\Delta Sr = 32.4 + 0.146(\Delta 8/4)$, $r = 0.85$; and $\Delta 7/4 = -0.86 + 0.127(\Delta 8/4)$, $r = 0.69$.

apparently characterized by unusually low ΔSr and negative values of Δ7/4 and Δ8/4. There is a good correlation between the features of the isotopic anomaly patterns of Fig. 2 and the degree-2 features of the Earth's geoid[10]. The geoid shows negative 'caps' in the polar regions and a nearly continuous belt of positive values centred on the Equator. The degree-2 geoid has been related by Busse[11] to a quadrupolar convection regime in the mantle, with lower mantle upwelling centred on an equatorial axis of symmetry. The isotopic anomaly data also suggest a simple quadrupolar pattern, but one which is more nearly axisymmetric with the Earth's spin axis. Higher-degree features of the isotopic anomaly pattern, such as the antipodal St Helena-type lows, have no obvious counterpart in the geoid.

Note that some complexities exist in the isotopic data for Hawaii and the Azores which have been obscured here by using averaged data. The Koolau series, Oahu[12] and the eastern end of Sao Miguel, Azores[6,13,14], show rather prominent Dupal signatures, with ΔSr = 41 and 53, and Δ8/4 = 51 and 44, respectively.

Implications

In evaluating models for the origin of this anomaly, several features need to be considered: (1) the fact that the anomaly is present in both OIB and MORB data (at least in the Indian Ocean); (2) the approximate coherence of the three anomaly criteria; and (3) positive values of the anomaly criteria require high time-integrated values for the parent/daughter ratios in the source (in the case of Δ7/4, the required high U/Pb value must, in addition, date back to early in Earth history), and (4) evidence for the Dupal signature in the past geological record. These points will be considered individually.

(1) Dupré and Allègre[1] proposed that the MORB reservoir (upper mantle) is polluted by material from the OIB reservoir (lower mantle), thereby transferring the anomaly signature from the lower to the upper mantle without creating complete isotopic identity between the two reservoirs. One can also conceive of a non-layered mantle scenario, where the OIB reservoirs are blobs distributed through the MORB reservoir and where partial cross-pollution takes place during partial melting and subsequent high-level magmatic processes[3,15].

Another explanation would relate these two reservoirs through a common parentage. If the depleted MORB reservoir is created by extraction of continental crust[16–19], and if the Dupal anomaly signature predates the formation of the MORB reservoir, then the existence of the anomaly signature in the MORB reservoir could simply be inherited from its mantle precursor. To be viable, this mechanism would require that the various MORB and OIB reservoirs have maintained their relative lateral positions over long periods of geological time, which seems unlikely in view of the rapid convective motions of the upper MORB reservoir. I prefer the 'pollution' explanation advanced by Dupré and Allègre[1].

(2) As shown in Fig. 1, the three anomaly criteria are reasonably, though not perfectly, coherent (r ranges from 0.69 to 0.85). Each anomaly criterion depends essentially on the time-integrated parent/daughter ratio in the reservoir; their coherence therefore suggests that the Dupal reservoir has higher time-integrated values of Rb/Sr (ΔSr), Th/Pb (Δ8/4) and $^{235}U/Pb$ (Δ7/4) than the reference reservoir. Because of the coupling of U/Pb and Th/Pb in the $^{208}Pb/^{204}Pb$–$^{206}Pb/^{204}Pb$ plot, the high Δ8/4 also requires high Th/U ratios. In other words, the Dupal source is characterized by high relative Rb/Sr, $^{235}U/Pb$ and Th/U; in most petrogenetic processes, the numerator element in these ratios is observed to behave more incompatibly than the denominator element, so that the approximate coherence of the three anomaly criteria is not surprising.

(3) The Dupal reservoir is characterized by relatively high time-integrated values of Rb/Sr, $^{235}U/Pb$ and Th/U. What does this imply for development of the Dupal reservoir? One obvious source for high Rb/Sr, U/Pb and Th/U material is the continental crust, and in fact, Dupré and Allègre[1] favoured the subduction of sediments into the mantle to explain the anomalous isotopic characteristics of the Indian ocean Dupal island suite. Other authors[3,20–22] have argued for reinjection of oceanic crust or sediment to provide a component in many or all OIBs.

Recent (<500 Myr) injection of crustal material is not a satisfactory mechanism for production of the Dupal isotopic signature, as this would produce high angle Pb isotope arrays, with an apparent origin somewhere along the Northern Hemisphere reference array. With the exception of Tristan, intra-suite Dupal trends appear instead to be subparallel to the reference array (for example, Gough, Walvis, Bouvet, Kerguelen). Furthermore, the suites showing the most marked Dupal signature define an inter-suite Pb–Pb array which is parallel to the reference array, but shifted to higher $^{207}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ values. This array, which is defined by 90° E-ridge—Discovery Seamount, Gough, St Paul, Crozet and Amsterdam—covers a large range in $^{206}Pb/^{204}Pb$ (17.9–19.2), and cannot be generated by contamination of reservoirs lying along the reference array with crustal material, even if that were heterogeneous. Inclusion of Sr isotope data only enhances the implausibility of this mechanism, as $^{87}Sr/^{86}Sr$ shows no significant correlation with position along this 'upper bound' array.

This problem can be circumvented if the crustal contaminant was reinjected into the mantle a long time ago, before the processes which have subsequently produced the 'lineation' of the reference suite along an apparent 1,700-Myr isochron, and which are also responsible for the subparallel lineation of the Dupal suites. Several reinjection models[3,21,22], in fact, propose that ancient oceanic lithosphere is responsible for the isotopic signature of many OIB suites, though it has yet to be demonstrated that such material will have the requisite high U/Pb and Th/U ratios. Furthermore, enhanced U/Pb ratios in altered oceanic crust should be accompanied by enhanced $U/^{3}He$ ratios, which would lead in several billion years to markedly enhanced $^{4}He/^{3}He$ ratios; there is no obvious difference in He isotope ratios between Dupal and non-Dupal islands[23].

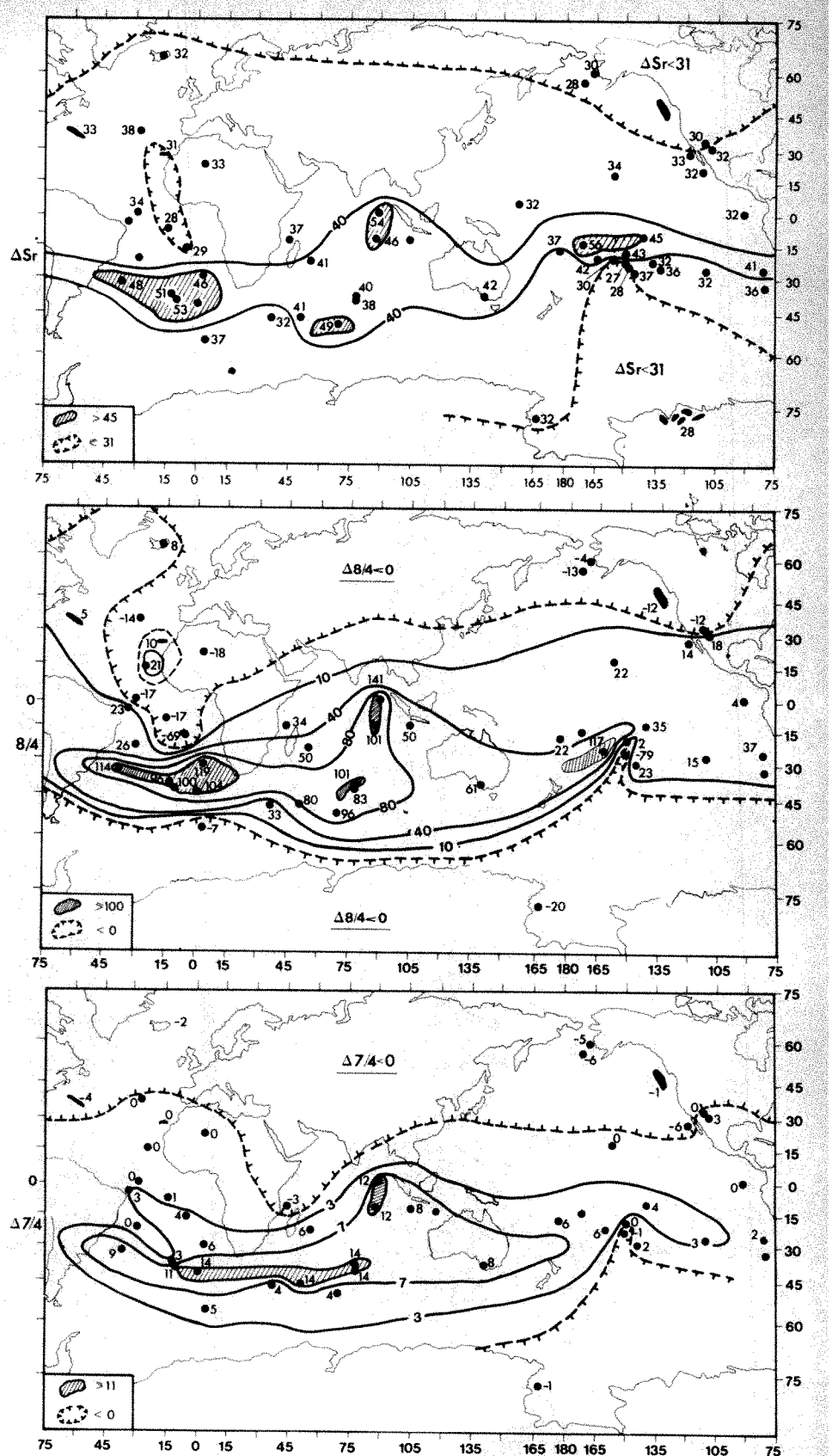


Fig. 2 World maps (Miller cylindrical projection) showing distribution of the ΔSr (upper), Δ8/4 (middle) and Δ7/4 (lower) anomaly criteria; contours are fitted by eye and are subjective in many areas of non-existent data. Anomaly data are from Table 1, augmented, in the case of ΔSr, by data from the Pacific Ocean[2,5] and Antarctica[46]. Also shown on all three maps are data for the Ninety-East Ridge[1], although these are samples of older age and should be plotted at a more southerly position consistent with their location of origin.

It has also been suggested that ancient sub-continental lithosphere may delaminate and provide the necessary 'enriched' reservoir[24]. This mixing would have to have occurred early in Earth history for the reasons discussed above. In any event, these reinjection models will need to address the fact that the Dupal anomaly is a coherent geographical domain, and is not randomly dispersed amongst the various OIB locations.

I present the following alternative hypothesis to the reinjection model of the Dupal anomaly, as a stimulus for further investigation. First, the anomaly is related to very early (>3,000 Myr) development of U/Pb, Rb/Sr and Th/U enrichments in the Dupal source mantle relative to the reference mantle. Second, this relative enrichment either derives directly from constructional heterogeneties in the initial Earth, or is related to heterogeneties developed during early stages of continental crust extraction. (More crust was extracted from the reference mantle reservoir, causing it to be more depleted than the Dupal mantle reservoir.) Third, in common with most other oceanic Pb models,

Table 1 Sr and Pb isotope ratios, averaged by island or area

	$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$	$^{87}Sr/^{86}Sr$	Δ7/4	Δ8/4	Ref.
Ahaggar	19.915	15.653	39.525	0.70328	0.3	−17.9	29
Amsterdam (1)	19.170	15.708	39.810	0.70400	13.9	100.6	1
Ascension	19.568	15.625	39.112	0.70282	1.3	−17.3	3
Azores	19.731	15.626	39.341	0.70379	−0.4	−14.1	3
Bouvet (3)	19.445	15.652	39.065	0.70369	5.3	−7.1	3
Canaries	19.600	15.613	39.419	0.70307	−0.3	9.6	3
Cape Verde (2)	19.080	15.560	38.900	—	0.1	20.5	30
Christmas	18.896	15.616	38.972	—	7.7	50.0	*†
Comores (2)	19.300	15.549	39.300	0.70368	−3.4	33.9	1
Crozet (1)	18.913	15.679	39.290	0.70411	13.8	79.7	1
Discovery (1)	18.246	15.609	38.723	—	14.0	103.6	30
Easter (2)	19.200	15.600	38.985	0.70320	2.8	14.5	3
Fernando d'Noronha (2)	19.501	15.631	39.429	—	2.6	22.5	30
Fiji	18.688	15.572	38.442	0.70369	5.5	22.1	4
Galapagos	18.953	15.547	38.586	0.70317	0.1	4.5	3
Gough	18.435	15.599	38.917	0.70532	11.0	100.2	3
Guadalupe	20.151	15.620	40.134	0.70326	−5.5	14.4	30
Hawaii	18.338	15.479	38.016	0.70338	0.0	21.8	3
Iceland	18.752	15.502	38.382	0.70317	−2.2	8.4	3
Kerguelen	18.188	15.503	38.573	0.70492	4.0	95.7	3
Marion-Prince Edward	18.570	15.548	38.412	0.70320	4.4	33.4	*‡
Marquesas	19.107	15.597	39.077	0.70451	3.5	35	8
NE seamounts	20.458	15.672	40.406	0.70329	−3.7	4.5	31
NE Pacific seamounts	18.575	15.492	37.966	—	−1.3	−11.8	32
Nunivak (1)	18.628	15.457	38.112	0.70297	−5.3	−3.6	33, 34
Potrillo (1)	18.541	15.530	38.223	0.70320	2.9	18.0	34, 35
Pribilofs	18.912	15.485	38.364	0.70284	−5.6	−12.8	36
Rapa (3)	19.051	15.578	38.892	0.70370	2.2	23.3	2, 9
Raratonga (2)	18.338	15.536	38.973	0.70420	5.7	117.5	2, 9
Reunion (2)	18.800	15.585	38.850	0.70410	5.6	49.4	1, 37
Rio Grande Rise (1)	17.619	15.490	38.073	0.70476	8.9	114.5	*§
Ross	19.870	15.634	39.450	0.70323	−1.1	−20.0	38, 39
San Carlos (1)	18.591	15.508	37.981	0.70298	0.2	−12.3	34, 35
San Felix (1)	19.312	15.602	39.349	0.70409	1.8	37.4	*‖
St Helena	20.804	15.789	40.091	0.70293	4.3	−68.8	3
St Paul (1)	18.684	15.653	39.050	0.70383	13.7	83.4	1
St Paul's Rocks (1)	19.773	15.638	39.360	0.70341	0.4	−17.3	40
Tahiti	19.006	15.547	38.624	0.70411	−0.4	1.9	5, 9
Trindade	19.069	15.559	38.945	—	0.1	26.4	41
Tristan da Cunha	18.551	15.527	39.013	0.70507	2.5	95.8	3
Tubuai	21.104	15.770	40.354	0.70277	−0.9	−78.8	8
Walvis Ridge	17.914	15.492	38.472	0.70457	5.9	118.7	42
W. Victoria	18.444	15.567	38.540	0.70425	7.7	61.4	43, 44

Also utilized are Sr isotope data on Gough, St Paul, Kerguelen, Amsterdam (S.R.H. and C. Brooks, unpublished data), and Kerguelen, Easter, Guadalupe, Gough, Tristan and St Helena[6]. Numbers in parentheses after locality are the least numbers of analyses involved in cases for which $n < 4$. Pb data from ref. 9 has been normalized to absolute values using California Institute of Technology Pb standard data of ref. 45.

* Unpublished data from S.R.H. and † J. Morris, ‡ A. J. Erlank, § H. Staudigel, ‖ D. Gerlach.

some means is required to produce the apparent secondary isochron Pb–Pb arrays observed in intra-island basalt suites, and in the reference reservoir suites as a whole. This requires a process, with a mean age of ~2,000 Myr, that will increase the U + Th/Pb ratio of all oceanic mantle sources. Core-pumping of Pb has been advocated for this[16], as has concentration of Pb into the lower crust[19]. Mixing with ancient high U/Pb oceanic crust (with present $^{206}Pb/^{204}Pb \geq$ that of St Helena) has also been advocated[3]. I have little to add to the problem of the Pb paradox, other than my prejudice that the Dupal anomaly predates, and is perhaps not even related to, the events which have created the oceanic Pb paradox.

(4) As outlined, the Dupal anomaly is globe-encircling and restricted to the Southern Hemisphere. I have argued above that this anomaly has probably existed as a mantle entity for billions of years.

Support for the antiquity of this anomaly comes from Sinha[25] who compiled existing Pb isotope data for continental rock and ore leads of all ages, and showed that samples from the Southern Hemisphere fall along a higher μ growth curve than did those from the Northern Hemisphere (closed system μs of 9.3 and 8.9 respectively). This distinction was present even in the oldest rocks considered (>3,000 Myr). This hemispheric distinction is not apparent in Pb data from conformable ore deposits[26,27] but many of these leads appear to be generated in an arc setting and may involve rather extensive mixing in sedimentary environments. Sinha did not compile $^{208}Pb/^{204}Pb$ data, so the existence of a Δ8/4 anomaly in this continental data set is not verifiable at present.

Note that the rock Pb isotopic data sets compiled by Manhes *et al.*[28], also show the Sinha divergence. The eight Southern Hemisphere data sets have a higher average $^{207}Pb/^{204}Pb$ intercept value than do the 12 Northern Hemisphere data sets. These sets consist of data of variable analytical quality, and represent a diversity of rock types with both crustal and mantle affinities. If the Sinha hemispheric Pb divergence withstands further scrutiny, two important conclusions are implied: (1) if this divergence is genetically related to the Dupal anomaly, then the Dupal anomaly is indeed of ancient constitution and; (2) the existence of a Dupal-type anomaly in continental rocks suggests a genetic link between oceanic island mantle and continental crust. Because the continental crust is presumed to derived from the mantle, it could carry the Dupal signature with it; on the other hand, if the Dupal anomaly originated by the ancient reinjection of continental material, the signature may have been originally bred in the Gondwana crustal entity and have been inoculated into the subjacent mantle during later crustal reinjection.

Future directions

The Dupal anomaly is defined on the basis of the present position of the oceanic islands and continents. If the anomaly is of ancient parentage where were these continental blocks and oceanic reservoirs in the past? Have they been anchored to the Southern Hemisphere? Could there be a mantle convection regime which would not, in billions of years, destroy the isotopic signature of the Dupal mantle, and which would not decouple the geographical positions of the Dupal continents and mantle reservoirs?

The next step is to define the Dupal anomaly more clearly by analysis of basaltic rocks from both oceanic and continental regimes in the Southern Hemisphere. It will also be useful to search for Dupal signatures in Southern Hemisphere volcanic arcs.

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Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences

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Two related, but distinct, cDNA clones have been isolated and sequenced from a functional murine cytotoxic T-lymphocyte clone. The genes corresponding to these cDNA are expressed and rearranged specifically in T cells and both have similarities to immunoglobulin variable and constant region genes. It is concluded that these genes code for the two subunits of the heterodimeric antigen receptor on the surface of the T cell; its complete deduced primary structure is presented.

THE most characteristic feature of the vertebrate immune system is its capacity to respond to an enormously diverse set of antigenic determinants. At the molecular level this capacity has been traced to an equally diverse set of glycoproteins that are synthesized in B and T lymphocytes. The glycoproteins produced by B cells, called antibodies or immunoglobulins, recognize and bind free antigens and are responsible for humoral immunity, while the T-cell molecules, commonly called T-cell receptors, recognize cell-bound antigens in the specific molecular context of self major histocompatibility complex (MHC) products[1–3] and are responsible for cellular immunity. The MHC-restriction is an acquired phenomenon[4,5] determined not by the genotype of the T cells but by that of the host thymus in which the T cells differentiate.

During the past several years extensive studies on the biology and chemistry of immunoglobulin molecules and their genes have illuminated structural and functional properties of these physiologically critical molecules and the genetic origins of their enormous diversity[6]. However, in spite of the equally central position they occupy in immunological phenomena, the chemical nature of the T-cell receptors has been elusive[7]. Only recently has the first glimpse of these molecules been obtained through the development of effective antisera and monoclonal antibodies that recognize and precipitate clone-specific proteins on the surface of functional T-cell clones, hybridomas or T-cell tumours[8–10]. These studies suggested that the portion of the receptor determining specificity is a heterodimeric glycoprotein corresponding to a molecular weight (M_r) of about 90,000 (90K) consisting of a 40–45K α-subunit and a 42–44K β-subunit. Moreover, peptide fingerprint analysis suggests that both the α- and β-subunits, like immunoglobulin heavy and light chains, are composed of variable (V) and constant (C) regions[11–13]. These studies, however, have not provided a primary structure because of the difficulty in preparing a sufficient amount of purified receptor protein.

More recently two groups of workers, using an entirely different approach, succeeded in isolating T-cell-specific cDNA clones of mouse or human origin which show significant

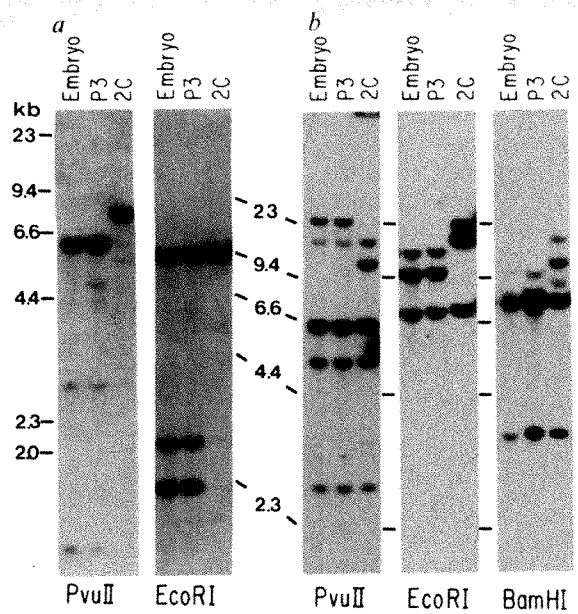


Fig. 1 Southern blot analysis of DNA from BALB/c embryos, myeloma P3 (BALB/c derived), and CTL 2C (BALB.B). DNA was digested with the indicated restriction enzymes, electrophoresed through 0.8% agarose, blotted onto nitrocellulose, and hybridized with ^{32}P-labelled, nick-translated inserts from clone pHDS11 (*a*) or clone pHDS4 (*b*). Hybridization was carried out at 42 °C in 50% formamide and 4×SSC. The filters were washed at 65 °C in 0.25% SSC. Separate experiments showed that BALB/c and BALB.B embryo patterns are indistinguishable.

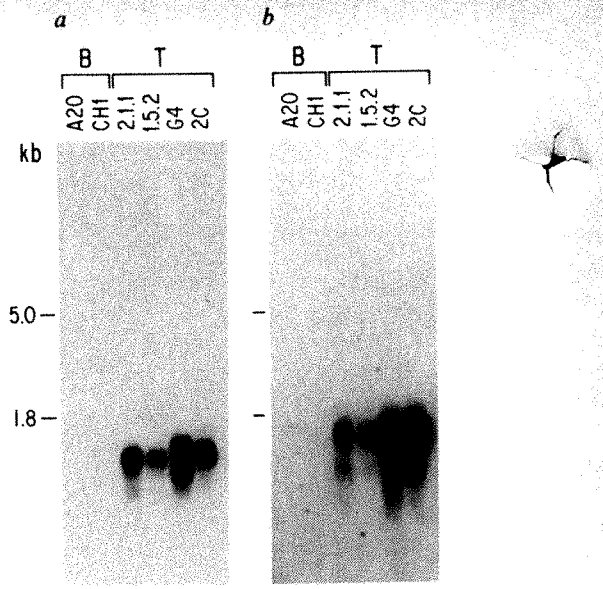


Fig. 2 RNA blot analysis of poly(A)$^+$ RNA from various B- and T-cell lines. RNA was extracted from B-cell lymphomas A20-2J and CH1 and alloreactive (H-2^b anti-H-2^d), cytotoxic T-lymphocyte clones 2.1.1, 1.5.2, G4 and 2C. Approximately 1.5 μg of poly(A)$^+$ RNA were denatured with glyoxal and electrophoresed through 1% agarose in 10 mM sodium phosphate buffer, *p*H 6.5. RNA was transferred to nitrocellulose and hybridized to ^{32}P-labelled, nick-translated inserts from clone pHDS11 (*a*) or clone pHDS4 (*b*). Positions of rRNA markers (5.0 and 1.8 kb) are as indicated.

homology to immunoglobulin genes[14–16]. Consistent with their interpretation that these cDNA clones represent one of the two subunits of the receptor, one group has shown that the cDNA encodes a protein composed of an amino-terminal V and a carboxy-terminal C region[16]. They also showed that the corresponding genomic DNA sequences have undergone clone-specific somatic rearrangements in various T-cell lines[15,16]. As the predicted amino acid sequences encoded by the mouse and human cDNA clones isolated by Hedrick *et al.*[16] and Yanagi *et al.*[14], respectively, are highly similar in the C regions, it is very likely that they represent only one of the two subunits of the T-cell receptor.

We have applied the subtraction-cloning method of Davis and his co-workers[15] to a clone of murine alloreactive, cytotoxic T lymphocytes (CTL) and have identified two clearly distinct, mutually non-cross-hybridizing T-cell-specific cDNA clones. Both of these clones show significant sequence homology to immunoglobulin light and heavy chains as well as to the putative receptor genes of Hedrick *et al.*[16] and Yanagi *et al.*[14]. In fact, one of our cDNA clones and those of Hedrick *et al.* have a virtually identical nucleotide sequence in the entire constant and 3′ untranslated regions. In addition, the genes corresponding to both types of our cDNA have undergone rearrangements in this as well as other cytotoxic T-cell clones.

On the basis of these findings we believe that we have identified and sequenced the genes coding for both subunits of the heterodimeric T-cell receptor of a functional CTL clone. We report here the predicted complete primary structure of this T-cell receptor.

Isolation of T-cell-specific cDNA clones

The alloreactive CTL clone 2C, of BALB.B origin and specific for products of the *D* end of the BALB/c *H-2* complex (*d* haplotype), has been described previously[17]. The cDNA synthesized on the poly(A)$^+$ RNA from 2C was subtracted twice with poly(A)$^+$ RNA from a mouse B-cell lymphoma, A20-2J (ref. 18), according to Hedrick *et al.*[15] and a library was constructed from the subtracted cDNA using the vector pBR322 and a standard G·C tailing method. A quarter of this library, containing about 20,000 independent cDNA clones, was subjected to differential screening using two hybridization probes. The first was the 2C cDNA prepared from the poly(A)$^+$ RNA of membrane-bound polysomes by subtraction with poly(A)$^+$ RNA from another B-cell lymphoma, CH-1 (ref. 19). The second was the cDNA prepared from the total poly(A)$^+$ RNA from A20-2J. One hundred and forty 2C-specific cDNA clones were identified and their plasmid DNA was used as hybridization probes in a series of RNA blotting experiments in order to confirm the T-cell-specific expression of the corresponding genes. This permitted us to group the T-cell-specific cDNA clones into at least 10 sets according to the sizes of the corresponding mRNA present in 2C. One was judged to be for the T-cell-specific surface marker Thy-1 on the basis of its hybridization to a previously identified Thy-1 cDNA clone (a gift of Mark Davis).

Two distinct cDNA classes

As was the case in the work of Hedrick *et al.*[15,16], one of our assumptions was that the receptor genes in question are rearranged in CTL. Thus we used representative cDNA clones of each set as hybridization probes and compared *Eco*RI-digested genomic DNA from 2C and BALB.B embryos by Southern blot analysis (data not shown). This screening led to the identification of two distinct classes of cDNA, one represented by clone pHDS11 and the other by clones pHDS4 and pHDS203, the genes of which are rearranged in 2C. The other classes of cDNA did not give any indication of DNA rearrangement.

Figure 1*a*, *b* shows the results of more extensive Southern blot analysis carried out using clone pHDS11 and pHDS4 as hybridization probes, respectively. The results can be summarized as follows. First, the hybridization patterns obtained by the

two probes are clearly distinct, confirming that the two classes of cDNA clones represent two different sets of genes that do not cross-hybridize to a detectable level in the conditions used. Second, with either probe the hybridization patterns obtained with 2C DNA are different from the patterns obtained with BALB embryo DNA. Third, the myeloma P3 DNA patterns are the same as the embryo DNA patterns. In addition, DNA from five different CTL clones was analysed and each gave patterns different from those of embryo DNA with at least one enzyme (data not shown). Considering the multiplicity and specificity of the pattern differences, these results strongly suggest that genes corresponding to both cDNAs have undergone gross sequence rearrangement in 2C.

The T-cell-specific expression of these genes has been confirmed by analysing poly(A)$^+$ RNA from 2C and three other independently derived alloreactive CTL as well as two B-cell lymphomas, A20-2J and CH1. As shown in Fig. 2*a*, *b*, each of the two cDNA probes detected RNA of distinct sizes in all four CTL but not in either of the B-cell lymphomas, although the relative content, and in some cases the size, of the RNA varied somewhat between CTL. When analysed with the clone pHDS11 probe, CTL 2C, 1.5.2 and 2.1.1 all gave a major RNA component of about 1.3 kilobases (kb) while CTL G4 contained two major components of 1.4 and 1.2 kb. On the other hand, all four CTL gave a 1.5 kb RNA component with the clone pHDS4 probe.

Nucleotide sequence analysis

The restriction maps of the three cDNA clones, pHDS11, pHDS4 and pHDS203, were constructed by standard procedures (Fig. 3), and the DNA sequences were determined by the method of Maxam and Gilbert[20] according to the strategy shown in Fig. 3. As both the restriction maps and DNA sequences (see below) confirm that pHDS4 and pHDS203 are derived from the same gene while pHDS11 represents a distinct one, we will describe the sequence features of the two genes separately.

The entire nucleotide sequence of the 1,054 base pair (bp) insert of clone pHDS11 is shown in Fig. 4*a*. The longest open reading frame is composed of 879 nucleotides whose corresponding amino acid sequence of 293 residues is also shown in Fig. 4*a*. For reasons given below, the codons are numbered starting with the triplet GAC at nucleotide positions 36–38. Between codons 110 (Glu) and 236 (Cys) the pHDS11 sequence is identical to the sequence of the corresponding region of Hedrick *et al.*'s thymocyte cDNA clone, 86T1 (ref. 16), except for one base pair difference in codon 159 (Arg). According to these authors this region defines the major body of the C region: codons 109 (Leu) and 110 (Glu) define the joining (J) and C region boundary while codon 236 (Cys) precedes the N-terminus of the transmembrane segment. The sequence identity is even more striking between pHDS11 and 2B4.71, the latter being a cDNA clone isolated from a T-helper cell hybridoma specific for pigeon cytochrome *c* (ref. 21). From codon 109 (Leu) the two sequences are virtually identical throughout the C regions and the entire 3′ untranslated regions. The exceptions are two base pair differences, one in codon 159 (Arg) and the other at nucleotide position 992.

Between codons 97 and 109 the pHDS11 sequence is similar to but distinct from the corresponding sequence of 86T1 or 2B4.71. As pointed out by Hedrick *et al.*[16], this region is strikingly homologous to the *J* segments of immunoglobulin genes. In fact the pHDS11 *J* sequence corresponds exactly to the sequence of the J_T7 genomic segment recently identified and characterized by Chien *et al.*[21].

Between codons 1 and 97 the pHDS11 sequence is quite different from the sequence of the corresponding region of either 86T1 or 2B4.71 (Fig. 5*a*). However, patches of conserved residues clearly exist throughout the three sequences and also between these sequences and those encoding immunoglobulin V regions of both light and heavy chains (the latter not shown). Particularly noteworthy conservations are the two Cys residues that are involved in intra-domain disulphide linkages in immunoglobulin V regions (residues 23 and 91 in Figs 4*a* and 5*a*). The Trp residue that is universally conserved in immunoglobulin V regions is also present in the corresponding position of the T-cell proteins (residue 34).

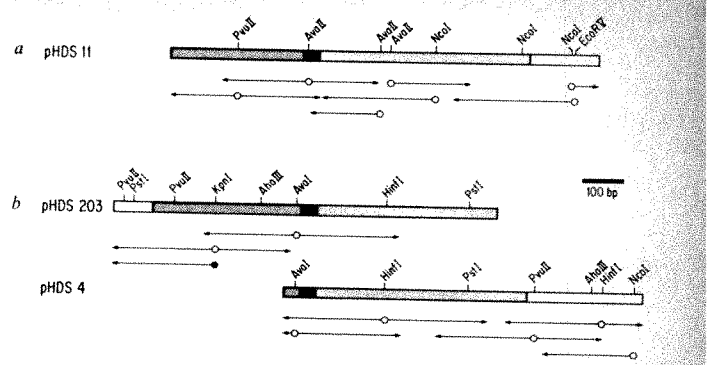


Fig. 3 Restriction maps of the inserts of cDNA clones isolated from CTL 2C. The maps were constructed by the standard single, double or triple digestions of the plasmid DNA. The *V*, *J*, *C* and 5′- or 3′-untranslated regions are shown by ▩, ■, ▨ and □, respectively. Also shown are the sequencing strategies used to produce the nucleotide sequences shown in Fig. 4. The 5′- and 3′-end labellings are indicated by ○ and ●, respectively.

At the 5′ end of the open reading frame (see Fig. 4*a*) there is a stretch of 11 highly hydrophobic residues that probably comprise part of a signal peptide[22]. The position of the N-terminal residue of the mature protein is ambiguous. However, homology between the pHDS11 protein and immunoglobulin V regions, particularly V_κ regions[23], suggests that Asp at position 1 is the N-terminal residue.

As already pointed out by Hedrick *et al.*[16], another stretch of a highly hydrophobic region of about 22 residues, evidently constituting a transmembrane peptide, occurs immediately before the C-terminal 5 hydrophilic residues; the latter are thought to extend into the cytoplasm. Overall, the gene defined by pHDS11 can encode a processed protein of 282 residues with a molecular weight of 32K.

The composite nucleotide sequence of 1,286 bp defined by the two overlapping cDNA clones pHDS4 and pHDS203 is shown in Fig. 4*b*. The longest open reading frame begins with the Met codon at nucleotide positions 88–90, extends over a stretch of 912 bp, and ends at nucleotide position 999. The predicted amino acid sequence is also shown in Fig. 4*b*. This sequence is significantly homologous to those of pHDS11, 86T1, 93G7 immunoglobulin heavy chain, MOPC603 immunoglobulin κ light chain, and MOPC104E immunoglobulin λ1 light chain. This is shown in Figs 5*b* and 6 where the pHDS4/203 protein sequence is compared with the V (or V+D), J and C region sequences of the other five proteins. Homology is evident in all three regions. The relatedness of the pHDS4/203 protein to the other five proteins in each of the three regions ranges over 18–23% for the V or V+D region, 21–50% for the J region and 16–22% for the C region. The similarity occurs in patches and in the case of the V (or V+D) region these patches tend to correspond to the framework regions (FR) of immunoglobulin V regions. In addition, many of the residues shared by the five non-pHDS4/203 proteins are also shared by the pHDS4/203 protein. These residues (indicated in Fig. 5*b* by *) include the Cys residues involved in the intra-domain disulphide linkages as well as the Trp residue which is universally present at the boundary of the first hypervariable (HV-I) and FR-2 segments of all immunoglobulin V regions studied to date[23].

As in the pHDS11 protein, the peptide at the amino-terminal end of the pHDS4/203 protein is highly hydrophobic and most probably constitutes a signal peptide. Accordingly the N-terminal residue of the processed protein cannot be unambiguously determined from the cDNA sequence. However, we believe that the N-terminal residue is the Gln at position 1 in Figs 4*b* and 5*b*. This is because the N-terminal residue of the α-subunit of the T-cell receptor is blocked, probably by a pyrollidone carboxylic acid residue (S. Schlossman, personal

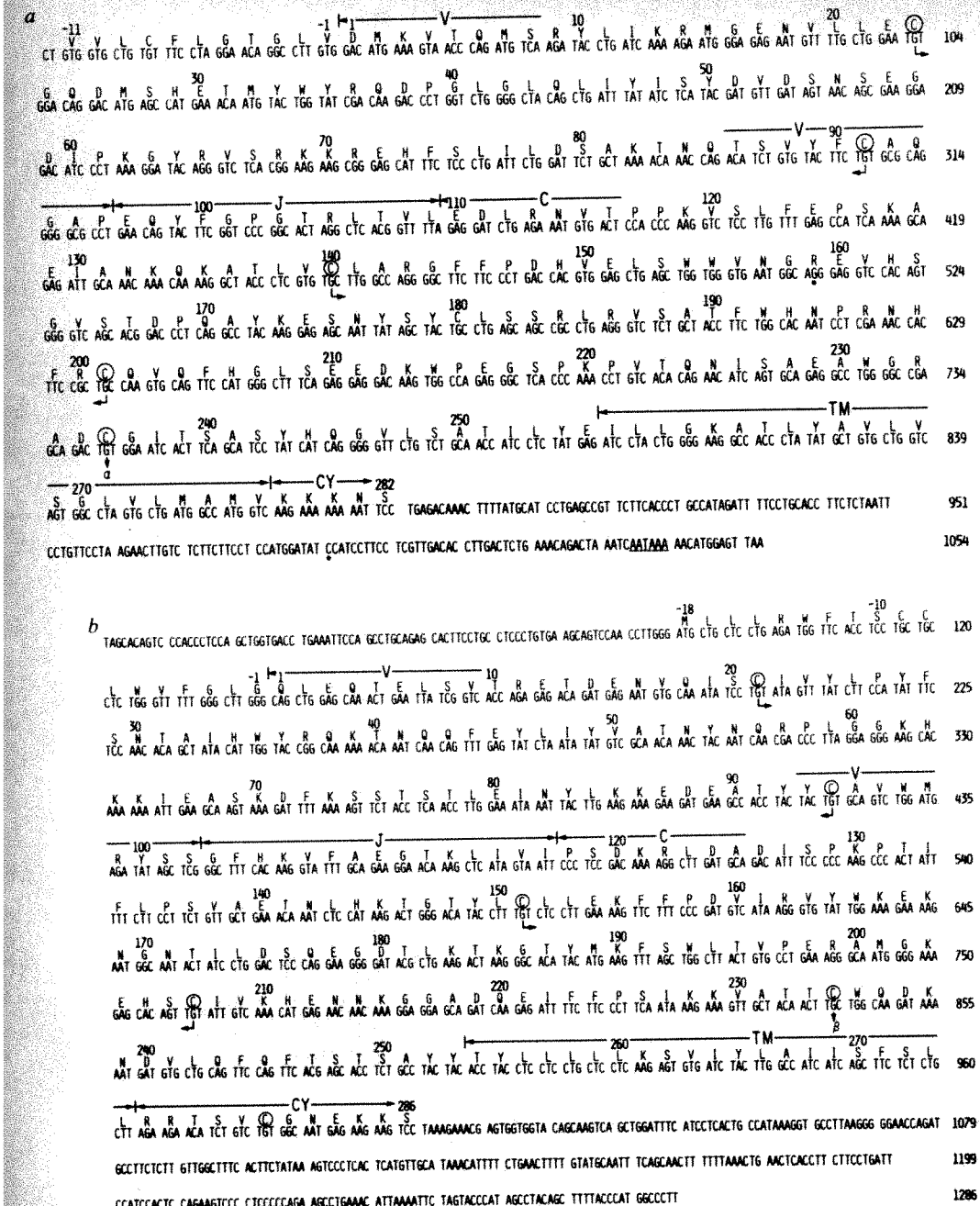


Fig. 4 The determined nucleotide and predicted amino acid sequences of cDNA clones pHDS11 (*a*) and pHDS4/pHDS203 (*b*). The numbers given above the amino acid sequences designate the residue positions. The numbers on the right show nucleotide positions. The negative numbers are for predicted signal peptides. The V, J, C, TM (transmembrane) and CY (cytoplasmic) regions are indicated although the exact boundaries are somewhat ambiguous. The cysteines thought to be involved in intra-domain or inter-chain disulphide linkages are indicated. The two nucleotides that are different in the C or 3′-untranslated regions of pHDS11 and 2B4·71 (ref. 21) are indicated by *. The oligonucleotide thought to be a signal for the poly(A) attachment site is underlined in *a*.

communication). In addition, this residue number assignment places the first conserved Cys residue at position 21, which is similar to the position (22 or 23) of the corresponding Cys residues in immunoglobulin regions[23].

Near its carboxy-terminus the pHDS3/203 protein also contains the second highly hydrophobic peptide of about 20 residues reminiscent of a transmembrane peptide. This region is followed by a hydrophilic C-terminal segment of 12 residues which probably comprises an intracytoplasmic peptide. The processed protein is 286 residues long and the calculated molecular weight is 33K.

The α- and β-chain

On the basis of T-cell-specific expression, T-cell-specific rearrangement and sequence similarity to immunoglobulin chains, the cDNA clones reported by Hedrick *et al.*[16], Yanagi *et al.*[14] and Chien *et al.*[20] have been thought to code for one subunit of the murine and human T-cell receptor. That this subunit is the β-chain has recently been shown by E. L. Reinherz and his co-workers (personal communication). They have purified the β-subunit of the receptor from a human T-cell tumour REX and determined the amino acid sequence of the N-terminal region. This sequence matches well with the N-terminal amino acid sequence predicted from the nucleotide sequence of the human cDNA clone of Yanagi *et al.*[14]. We have shown that pHDS11 gene is expressed and rearranged in CTL, but not in B-lymphoma cells, and that the C-terminal half of the protein encoded by this gene is similar or identical to the corresponding regions of the proteins encoded by the clones reported by Yanagi *et al.*[14], Hedrick *et al.*[16] and Chein *et al.*[21]. Thus we can conclude that clone pHDS11 encodes the β-subunit of CTL 2C.

The gene defined by cDNA clones pHDS4 and pHDS203 is also expressed and rearranged specifically in T cells. This gene and the β-chain gene are clearly distinct and do not cross-hybridize in standard conditions, although they are related. At the protein level the pHDS4/203 sequence is approximately as closely related (16–23%) to immunoglobulin light and heavy chains as is the β-chain. About the same level (20%) of sequence identity exists between the pHDS4/203 protein and the β-chain. This relatively low overall sequence similarity, however, is somewhat misleading in that it overlooks the striking organizational similarity between the two proteins: each of them contains two

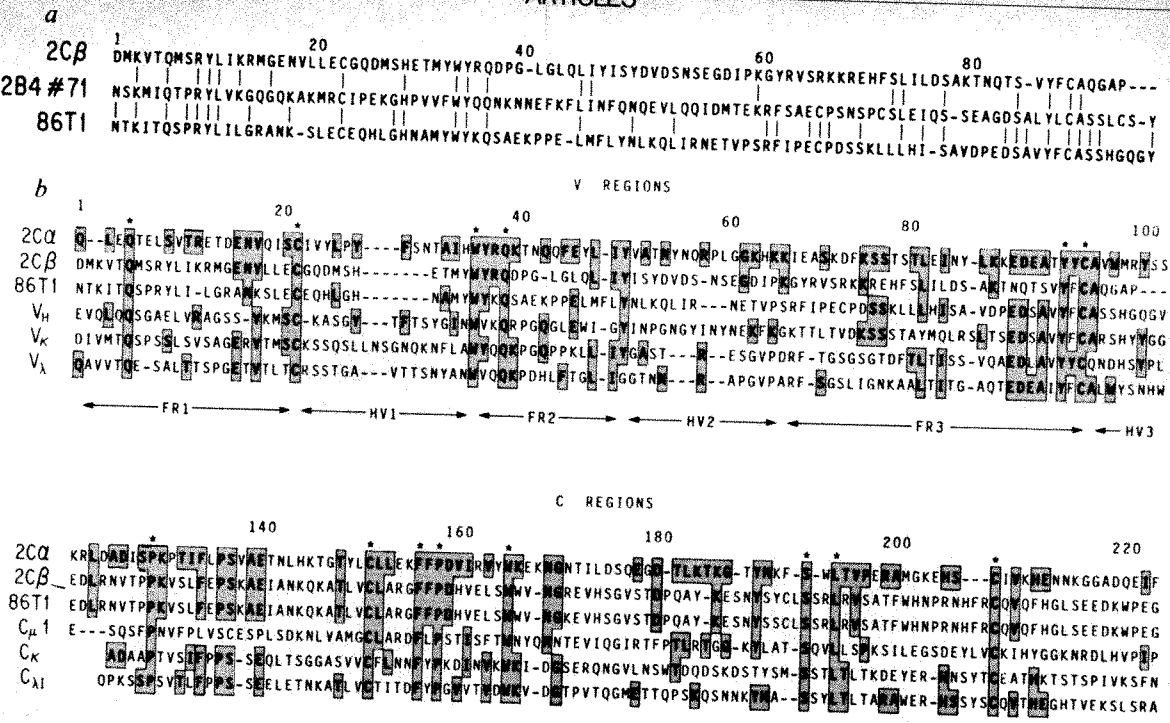


Fig. 5 *a*, Comparisons of the predicted amino acid sequences of three V_β regions, 2C β (pHSD11), 2B4.71 (ref. 21) and 86T1 (ref. 16). Those residues common between 2C β and 2B4.71, or 2B4.71 and 86T1 are indicated by vertical lines. *b*, Comparisons of the predicted amino acid sequence of pHDS4/203 (2C α) and predicted or determined amino acid sequences of five other proteins, the β-chain encoded by pHDS11, the β-chain encoded by cDNA clone 86T1 (ref. 16), the V regions of the 93G7 γ1 heavy chain[23], the C_H1 region of the μ heavy chain, the V and C regions of the MOPC603 κ chain[23] and the MOPC104E λ1 chain[23]. Those residues common between the 2C α polypeptide chain and any of the other five chains are shaded. The residues common among all six chains are indicated by *. Approximate boundaries of framework and hypervariable regions as they appear in immunoglobulin V regions are indicated by arrows.

immunoglobulin-like domains, a transmembrane peptide and a cytoplasmic peptide of similar sizes in the two chains. Earlier studies at the protein level indicated that the mouse receptor is composed of two different subunits of similar size held together by one or more disulphide linkage(s)[8–10]. In agreement with these findings, the predicted molecular weight (33K) of the pHDS4/203 protein is very similar to that (32K) of the β-chain encoded by pHDS11. Also, the pHDS4/203 protein and the pHDS11 β-chain each contain one Cys residue in corresponding positions (234 and 236, respectively) that lie outside the immunoglobulin-like domains. These residues can provide the postulated inter-chain disulphide linkage. Thus, while direct evidence is yet to be produced it is very likely that pHDS4/203 codes for the α-subunit of the CTL receptor.

Proposed structure of the T-cell receptor

The amino acid sequences deduced for the two subunits suggest the overall structure for the T-cell receptor shown in Fig. 7. In this model each receptor molecule is made up of two chains, each with two extracellular immunoglobulin-like domains, an amino-terminal variable and a carboxy-terminal constant domain. Each of these domains is stabilized by an S–S bond between cysteine residues that are separated in the linear sequence by 60–70 residues. Amino acid sequence similarity with immunoglobulin V regions (Fig. 5*b*) point to V (or V+D), D and J segments in the N-terminal domain of the α-subunit (Vα), as previously proposed for the corresponding domain of the β-subunit (Vβ)[16,21].

Cysteine residues at positions 234 and 236 of the α- and β-subunits, respectively, could well form a single inter-chain S–S bond located close to the cell outer membrane. This bond would link the two subunits in the intact molecule and account for the difference in apparent molecular weight between the unreduced and reduced receptor (90K compared with 40–45K) in SDS–polyacrylamide gel electrophoresis (PAGE). More important, a stable association between two different subunits may well be required to form an effective antigen-binding region, as is characteristic of immunoglobulins. The obligate participation of two different subunits in the formation of a single combining site means that combinatorial variability is likely to contribute to structural and functional diversity of these receptors.

The protein molecular weights of the α- and β-subunits are each about 10K less than the apparent molecular weight observed in SDS–PAGE. The difference suggests that the subunits are glycosylated. There are four potential sites for *N*-glycosylation of the β-subunit but none in the α-subunit. It is possible that the α-subunit is *O*-glycosylated on serine and threonine residues.

After the constant domain, each subunit has at its carboxyl end a hydrophobic stretch of 21–22 amino acids followed by a short stretch of 5(β) or 12(α) amino acid residues in which cationic residues abound. As these segments correspond, respectively, to the transmembrane and cytoplasmic domains that are characteristically found in transmembrane proteins, they support the proposed membrane location and receptor function of the gene products.

The presence of a cysteine residue in the cytoplasmic domain of the α-subunit might provide an SH group for dynamic

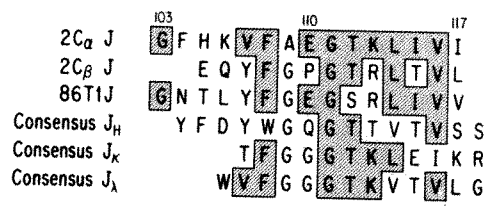


Fig. 6 Comparisons of the J region sequence of 2C α (predicted from pHDS4/203) and those of 2C β (predicted from pHDS11). 86T1 (ref. 16), and immunoglobulin J_H, J_κ, and J_λ (ref. 23) consensus sequences. Those residues common between 2C α J and any one of the other five J segments are boxed in and shaded.

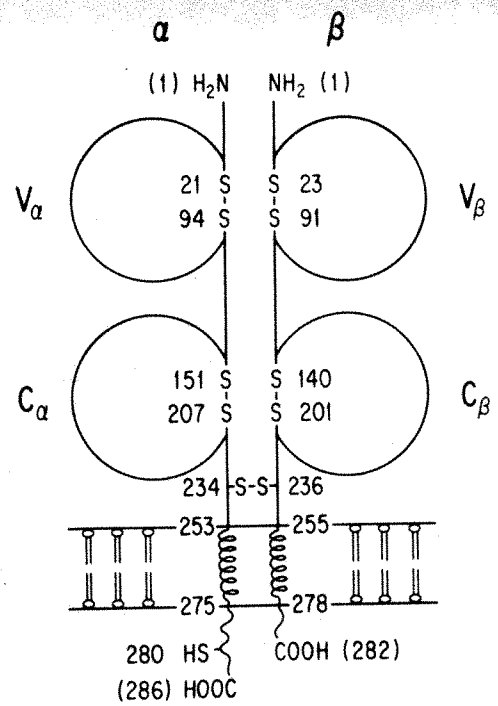


Fig. 7 Proposed overall structure of the 2C receptor. Each receptor molecule is made up of two chains, α and β, each with two extracellular immunoglobulin-like domains, an amino-terminal variable and a carboxy-terminal constant domain. Each of these domains is stabilized by an S–S bond between cysteine residues. Two chains are held by a single inter-chain S–S bond located close to the cell's outer membrane. The protein is anchored on the membrane by two (one for each chain) hydrophobic transmembrane peptides. A short carboxy-terminal peptide rich in cationic residues extends into cytoplasm in each chain. The SH group of the cystein residue present in the cytoplasmic peptide of the α-subunit may interact dynamically with a membrane or cytoplasmic protein and thereby may be important for the cell's effector function. Both α- and β-chains are glycosylated, although the exact site(s) and extent of glycosylation are unknown.

interactions between the receptor and other molecules of the cell membrane (such as T3 (ref. 9) in human CTL) or perhaps the cytoskeleton. The need for a functional association with such accessory structures is suggested by the overall resemblance of the proposed model to Fab fragments of immunoglobulin molecules: as is well known, these fragments have ligand-binding activity but lack effector functions.

These data throw light on the question of whether CTL and T-helper cell receptors are encoded by the same or separate sets of genes. We will consider the V and C regions separately because the two regions are encoded by distinct gene segments (ref. 21 and our unpublished observations). In immunoglobulin molecules the primary function of the V_H and V_L regions is, of course, to determine antigen specificity. Each of the multiple C_H regions defines an immunoglobulin class and carries a distinct effector function. The primary function of the C_L region is unclear. It is not required for antigen specificity but may be necessary to enhance L–H gene interactions or to facilitate some unknown function. In T-cell receptors the very presence of the V regions strongly suggests that these regions have a primary role in determining antigen and MHC specificity. An interesting question is whether the CTL and T-helper cell receptors carry distinct C regions each of which has a unique role in the activation or execution of the cell's effector function. An alternative view is that the receptors of the two types of T cells have the same C regions and that while the $\alpha\beta$ heterodimer is specific with regard to ligand binding it has only a nonspecific role in triggering the cell's effector functions.

Comparison of the β cDNA (clone pHDS11) from CTL clone 2C and the β cDNA (clone 2B4.71) from T-helper cell clone 2B4 indicates that at least these CTL and T-helper cells use the same C_β gene segment (clone 2C and clone 2B4 are from the BALB.B and B10.A strains, respectively, and the single base pair differences between their cDNAs may be due to a strain difference.) This conclusion is supported by two observations. First, the near identity of the two cDNA sequences occur not only in the C regions but also in the 3′ untranslated regions. Second, the J segments in the two cDNAs are different but belong to the same cluster linked to a single C gene segment[21]. While sequencing of more β genes is required before a firm conclusion can be drawn, these results already suggest strongly that the same C gene segments are used for β chains of CTL and T-helper cells.

Concerning the V regions, several earlier observations suggested that CTL and T-helper cell receptors use non-overlapping sets of gene segments that have evolved separately under different selective pressure: first, the antigen repertoires seem to be quite different; second, different classes of MHC gene products restrict CTL and T-helper cell responses; third, no clearly demonstrated case of T-cell reactivity that crosses the MHC class barrier has been reported (D. Raulet, personal communication). An alternative view is that CTL and T-helper cell receptors share a common pool of germ-line gene segments for V regions and that the distinction between function arises by somatic selection.

The amino acid sequences predicted by the 2C β cDNA (clone pHDS11) and 2B4 β cDNA (clone 2B4.71) are only similar at 21% of residues in the V (or $V+D$) segment-encoded regions (Fig. 5a). This is substantially lower than the similarity observed between any pair of immunoglobulin V_k segments (40–98%)[23] and is even less than that observed between the C_β region and the immunoglobulin C_k or $C_{\lambda 1}$ domain (22% and 31%, respectively)[16]. While characterization of more V_β regions is required before a firm conclusion can be drawn, the weakness of the similarity between the CTL V_β and T-helper cell V_β regions is striking and may reflect independent pools of V and/or D gene segments.

Finally, it will be extremely interesting to compare in similar fashion the V_α and C_α regions of CTL and T-helper cells. It is possible that, as in immunoglobulin molecules, the C region of only one of the two types of chains in these antigen-recognition molecules is involved in effector function.

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Molecular cloning of cDNA encoding a murine haematopoietic growth regulator, granulocyte–macrophage colony stimulating factor

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cDNA clones specifying the murine granulocyte–macrophage colony stimulating factor have been isolated. This haematopoietic growth factor is encoded by a unique gene specifying a messenger RNA of 1,200 nucleotides and a polypeptide of 118 amino acids. It bears no structural similarity to the functionally related factor, interleukin-3, described recently.

INDIVIDUAL multipotential stem cells can give rise to all of the different cell types of the haematopoietic and lymphoid systems[1]. Although this process is poorly understood, it has become apparent from *in vitro* studies that the proliferation, differentiation and functional activation of the various blood cells are controlled by a group of glycoprotein regulator factors including erythropoietin, T-cell growth factor (IL2) and the colony stimulating factors (CSFs)[2]. The colony stimulating factors which regulate the formation of neutrophilic granulocytes and macrophages have distinctive biological activities (M-CSF is a selective proliferative stimulus for macrophages[3]; G-CSF for granulocytes[4]; GM-CSF for both granulocytes and macrophages[5]), while multi-CSF—also known as interleukin-3 (ref. 6), burst-promoting activity[7], P-cell stimulating factor[8], mast cell growth factor[9] and haematopoietic cell growth factor[10]—stimulates the proliferation of not only neutrophilic granulocytes and macrophages, but also eosinophils, megakaryocytes, erythroid and mast cells[11]. Although it has been possible to purify all four colony stimulating factors, detailed analysis of many aspects of their biology and biochemistry has been hampered by the limited quantities available. This problem can be largely circumvented by molecular cloning of the corresponding gene sequences.

The present report describes molecular clones corresponding to GM-CSF. GM-CSF synthesized by mouse lung tissue is a glycoprotein of molecular weight 23,000 (ref. 5) which is active at low concentrations (10^{-11}–10^{-12} M) and is required continuously for the *in vitro* proliferation of progenitor cells of granulocytes and macrophages[2]. It also controls the irreversible commitment of these progenitors to the formation of granulocytes and macrophages[12] and can regulate the functional activity of the mature end cells[2]. A partial NH_2-terminal amino acid sequence has recently been determined for GM-CSF isolated from mouse lung-conditioned medium (L. Sparrow, D.M., T. Hunkapillar, L. Hood and A.W.B., manuscript in preparation). Using this partial amino acid sequence, oligonucleotides complementary to the putative GM-CSF mRNA were synthesized and used as probes to identify cDNA clones corresponding to the GM-CSF messenger RNA in a mouse lung cDNA library. From the nucleotide sequence of the cDNA clones, we deduced the amino acid sequence of GM-CSF and compared it with that recently reported for a functionally related growth factor, multi-CSF (interleukin-3)[13,14].

Isolation of GM-CSF clones

All mouse tissues contain and synthesize detectable amounts of GM-CSF, although the levels are extremely low[15]. To isolate a GM-CSF complementary DNA clone, we constructed a cDNA library using mRNA from the most active tissue available: lungs from mice injected with bacterial endotoxin[16]. The library was searched for GM-CSF clones by hybridization with synthetic oligonucleotides complementary to two short regions of the putative GM-CSF mRNA sequence, predicted from the partial NH_2-terminal amino acid sequence of the protein. We used oligonucleotide probes complementary to two different regions of the mRNA to help rule out irrelevant clones: whilst some clones may contain sequences fortuitously complementary to one of the oligonucleotide probes, a clone containing the GM-CSF sequence would be expected to hybridize to both. Figure 1 shows the GM-CSF amino acid sequence between residues 7 and 16, the possible combinations of nucleotide sequences that could encode this peptide and the oligonucleotides that were used as hybridization probes. As the assignment of amino acid 9 was equivocal (being either histidine or cysteine), two different sets of oligonucleotides encompassing this region were synthesized; probe 1 assumes a histidine and probe 2 a cysteine residue. The second region chosen within the amino acid sequence required an extremely degenerate set of oligonucleotides which were synthesized as two 48-fold degenerate sets (probes 3 and 4).

In the initial screening, all four sets of oligonucleotide probes were used as a mixture (see legend to Fig. 1); screening of ~100,000 cDNA clones yielded 22 positives, of which two (pGM37 and pGM38) hybridized separately with probe 1 and with a mixture of 3 plus 4 and were therefore strong candidates to contain sequences coding for GM-CSF. In order to confirm their identity, we asked whether transcripts complementary to these cloned sequences were present in cells that synthesize GM-CSF; whether DNA from these clones could specifically select, by hybridization, biologically active GM-CSF mRNA; and whether the nucleotide sequence of the clones could encode the known GM-CSF protein sequence.

Detection of GM-CSF mRNA

Initially we determined whether the abundance of transcripts corresponding to pGM38 in various cell types paralleled the ability of those cells to synthesize GM-CSF. Messenger RNA from mouse lung (which synthesizes GM-CSF) and from various cells which do not synthesize GM-CSF was fractionated on formaldehyde–agarose gels, transferred to nitrocellulose and hybridized with a probe derived from pGM38. Figure 2 shows that this probe detected a low abundance transcript of about 1.2 kilobases (kb) in mRNA from mouse lung (lane 3), but not in mRNA from several randomly selected cell lines which do not synthesize GM-CSF, including the thymoma-derived cell line TIKAUT (lane 1) and the plasmacytoma P3 (lane 2). Furthermore, this probe failed to detect any transcript in mRNA from WEHI-3B D^- (lane 6), RIII (lane 7) and L cells (lane 8), which synthesize multi-CSF[6], G-CSF (N.A.N., G. R. Johnson and R. H. Whitehead, unpublished results) and M-CSF[3] respectively. The very low abundance of the transcript corresponding

to pGM38 in lung mRNA was not surprising given the low frequency of pGM38-related cDNA clones in the lung cDNA library (~1 in 50,000).

As a more critical test of whether transcripts complementary to pGM83 correlated with GM-CSF production, we used a cloned T lymphocyte cell line (LB3)[17] in which the synthesis of both GM-CSF and multi-CSF is induced by concanavalin A (A.K., D.M. and A.W.B., unpublished results). Figure 2 shows that the pGM38-derived probe detected an abundant mRNA in LB3 cells that had been stimulated with concanavalin A (lane 5) but failed to detect any transcript in mRNA from unstimulated LB3 cells (lane 4). As the probe used does not hybridize to the multi-CSF mRNA in WEHI-3B D⁻ (lane 6), it is likely that pGM38 hybridized to the GM-CSF mRNA in LB3 cells.

Synthesis of GM-CSF in *Xenopus* oocytes

As a direct test of the identity of pGM38, we sought to establish whether this cDNA could specifically select, by hybridization, biologically active GM-CSF mRNA. GM-CSF mRNA was identified by translation in *Xenopus* oocytes and the culture medium assayed for ability to stimulate granulocyte/macrophage proliferation in three microculture systems: (1) liquid cultures containing purified fetal liver haematopoietic progenitor cells, which respond directly to all four colony stimulating factors[18,19]; (2) micro-agar cultures containing bone marrow cells, in which the formation of morphologically identifiable granulocyte and macrophage colonies can be stimulated by all four colony stimulating factors; and (3) liquid cultures containing the factor-dependent mast cell line, 32D C13 (ref. 20), which responds to multi-CSF but not GM-CSF[21]. From this set of assays, GM-CSF and multi-CSF can be unambiguously distinguished.

Initially we tested whether pGM38 DNA could select GM-CSF mRNA from mouse lung RNA. After injection of unfractionated lung mRNA or mRNA selected by hybridization to pGM38 DNA into *Xenopus* oocytes, very low levels of CSF activity could be detected in the culture media, a finding that was anticipated from the very low abundance of mRNA corresponding to pGM38 in mouse lung (Fig. 2). By contrast, mRNA from concanavalin A-stimulated LB3 cells (which synthesize both GM- and multi-CSF) is rich in mRNA corresponding to pGM38 (Fig. 2) and, on injection into *Xenopus* oocytes, reproducibly directed the synthesis of high levels of material stimulating the proliferation of fetal liver and 32D C13 cells (Fig. 3). Triplicate filters containing either pGM38 or vector DNAs were therefore challenged with concanavalin A-stimulated LB3 mRNA in a hybrid-selection experiment (see legend to Fig. 3). Figure 3*a* shows that medium from oocytes injected with mRNA selected by pGM38 DNA strongly stimulated fetal progenitor cell proliferation with the development of maturing granulocytes and macrophages (Fig. 4). These oocyte-conditioned media also stimulated typical[22] granulocyte–macrophage colony formation in micro-agar cultures (18% granulocyte, 53% mixed granulocyte–macrophage and 29% macrophage colonies). In neither assay was there evidence that these oocyte-conditioned media stimulated the proliferation of erythroid, megakaryocytic or eosinophilic cells, hence the active factor appeared to be GM-CSF rather than multi-CSF. In confirmation of this conclusion, these oocyte-conditioned media were found to be incapable of stimulating proliferation of 32D C13 cells (Fig. 3*b*). Vector DNA alone failed to select mRNA corresponding to GM-CSF or multi-CSF (Fig. 3*a*, *b*).

Taken together, these data indicate that pGM38 DNA can specifically select a mRNA encoding a factor with the unique biological characteristics of GM-CSF from a mixture containing both GM-CSF and multi-CSF mRNAs.

Nucleotide sequence of GM-CSF mRNA

Nucleotide sequence analysis of the cDNA portion of pGM37 and pGM38 revealed that these two clones, which contain sequences complementary to overlapping portions of the same mRNA, contain the putative GM-CSF mRNA sequence. The relationship between the cDNA portion of the two clones and

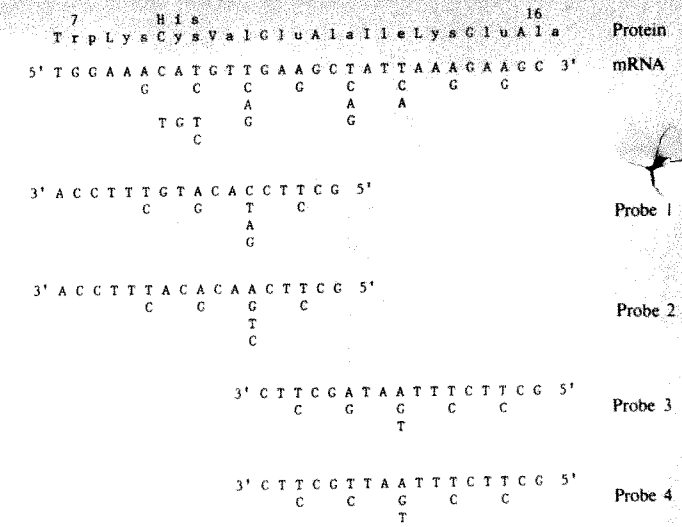


Fig. 1 Synthetic oligonucleotides used for identifying GM-CSF clones. A portion of the amino acid sequence of murine GM-CSF (residues 7–16) is shown on the top line, the possible combinations of nucleotide (mRNA) sequences that could encode this peptide segment in the middle, and the four different sets of oligonucleotide probes complementary to regions of this mRNA sequence below. **Methods:** The methods used for the construction and screening of the lung cDNA library were as follows. Ninety C57BL/6 mice were injected intravenously with bacterial endotoxin (5 μg per mouse) and after 3 h the lungs were removed and cultured *in vitro* in serum-free Dulbecco's modified Eagle's medium[16]. Total cellular RNA was extracted, essentially as described in ref. 42, from batches of 30 pairs of lungs after 0, 5 and 15 h of culture, pooled and then poly(A)-containing RNA prepared by oligo(dT)–cellulose chromatography. A duplex DNA copy of the pooled lung mRNA was synthesized using avian myeloblastosis virus reverse transcriptase for synthesis of the first-strand and *Escherichia coli* DNA polymerase I (Klenow fragment) for synthesis of the second strand, using standard techniques[43,44]. After cleavage of the hairpin loop with S_1 nuclease, oligo(dC) tails (approximately 20–30 residues per end) were added using terminal deoxynucleotidyltransferase[45]. The tailed cDNA was fractionated by electrophoresis on a 1.5% agarose gel and molecules greater than 500 bases in length recovered and annealed to dG-tailed plasmid DNA. The plasmid used (pJL3) is an SV40-based expression vector (J.G., N.M.G and A.R.D., manuscript in preparation) that contains the β-lactamase gene and origin of DNA replication from pAT153, the SV40 origin of DNA replication and T-antigen coding sequences and a multi-cloning site adjacent to the SV40 late promoter. *E. coli* MC1061 (ref. 46) was transformed with the annealed cDNA–plasmid mixture using a high efficiency transformation procedure (J.G., unpublished) that yields $>10^8$ transformants per μg of vector DNA. Pools containing approximately 2,000–3,000 ampicillin-resistant clones were stored in 10% glycerol at −70 °C. For screening by colony hybridization, approximately 4,000 bacterial colonies from each pool were grown on agar plates (containing 40 μg ml^{-1} ampicillin), transferred to nitrocellulose filter disks and plasmid DNA amplified by incubation of the filter on agar plates containing 200 μg ml^{-1} chloramphenicol[47]. After regrowth of colonies on the original plate, a second nitrocellulose filter was prepared. The master plate was regrown a second time and then stored at 4 °C. Plasmid DNA was released from the bacterial colonies and fixed to the nitrocellulose filters[19]. Before hybridization, filters were incubated for several hours at 37 °C in 6×SSC (SSC = 0.15 M NaCl, 0.015 M sodium citrate), 0.2% Ficoll, 0.2% polyvinyl-pyrrollidone (PVP), 0.2% bovine serum albumin (BSA) containing 50 μg ml^{-1} of denatured salmon sperm DNA and 10 μg ml^{-1} of denatured *E. coli* DNA. Hybridization was in the same solution, containing 0.1% NP40, at 37 °C for about 18 h. The synthetic oligonucleotides (kindly supplied by S. Horvath and L. Hood) were radioactively labelled using [γ-^{32}P]ATP and polynucleotide kinase and each was present in the hybridization reaction at 1.5 ng ml^{-1}. After hybridization, filters were extensively washed in 6×SSC, 0.1% SDS at 42 °C and then autoradiographed. Colonies positive on duplicate filters were picked and rescreened at low density as before.

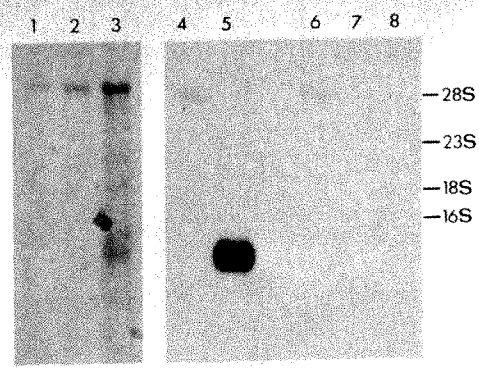


Fig. 2 Detection of GM–CSF mRNA in various cells. 5 μg of poly(A) RNA from TIKAUT (lane 1), P3 (lane 2), mouse lung (lane 3), unstimulated LB3 (lane 4), concanavalin A-stimulated LB3 (lane 5), WEHI3B D⁻ (lane 6), RIII (lane 7) and L cells (lane 8) were electrophoresed on 1% formaldehyde/agarose gels, transferred to nitrocellulose and hybridized with a fragment of DNA spanning the entire insert of pGM38 ^{32}P-labelled by nick-translation[48]. Hybridization was at 65 °C for 16 h in 2×SSC, 0.1% SDS, 0.2% Ficoll, 0.2% PVP, 0.2% BSA containing 50 μg ml^{-1} of denatured salmon sperm DNA. LB3 (formerly B3)[24] is a cloned Thy-1$^+$, Lyt-2$^-$, MT4$^+$ T lymphocyte line derived from BALB/c anti-DBA/2 mixed leukocyte culture, and maintained by weekly passage with irradiated DBA/2 spleen cells (1,500 R) in the presence of interleukin-2. Concanavalin A-stimulated LB3 cells were prepared by culturing cells at 10^6 per ml with 5 μg ml^{-1} concanavalin A in tissue culture medium with 5% fetal calf serum for 5 h. The molecular weight markers used and their presumed molecular weights were: mammalian 28 and 18S rRNA (4,700 and 1,800 nucleotides) and *E. coli* 23 and 16S rRNA (2,904 and 1,541 nucleotides). A low level of hybridization to residual 28S rRNA is evident after long autoradiographic exposure (tracks 1–3).

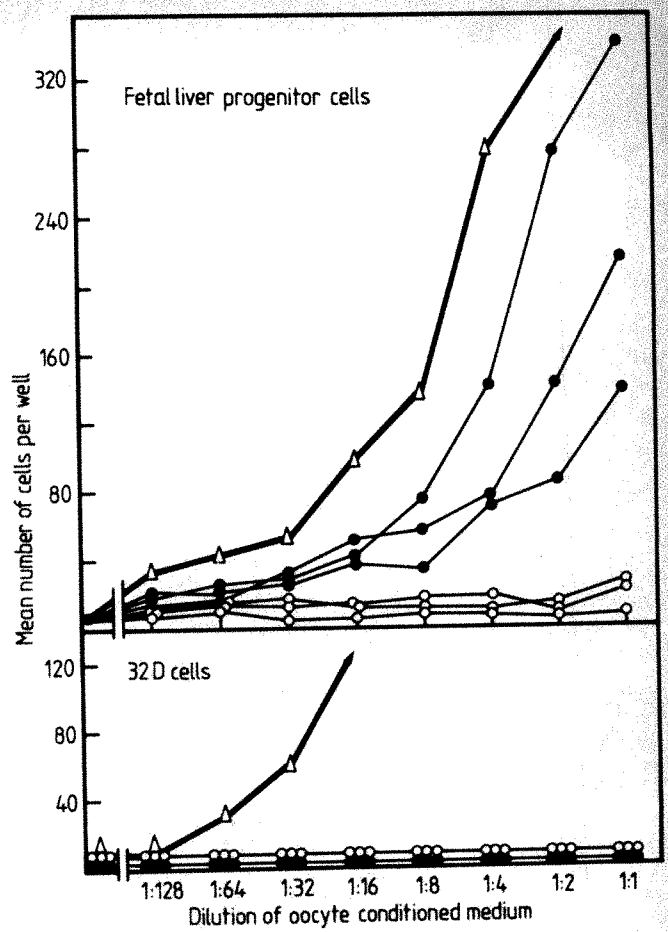


Fig. 3 Stimulation of cellular proliferation in suspension cultures of haematopoietic progenitor cells from fetal liver (upper panel) and multi-CSF-dependent 32D C13 cells (lower panel) by medium from mRNA-injected *Xenopus* oocytes. Unfractionated LB3 mRNA is indicated by heavy lines. ●—●, mRNA selected by hybridization to pGM38 DNA; ○—○, that selected by vector DNA alone; the three separate curves represent triplicate experiments. **Methods:** Hybrid selection was performed essentially as described elsewhere[49]. pGM38 or vector (pJL3) DNA (5-μg aliquots) were bound to small squares of nitrocellulose and incubated with 1–2 μg aliquots of concanavalin A-stimulated LB3 mRNA in 50% formamide, 0.5 M Tris-HCl *p*H 7.5, 0.75 M NaCl, 0.002 M EDTA, 0.4% SDS, 10 μg ml^{-1} *E. coli* tRNA in a reaction volume of 30 μl at 37 °C for 16 h. After incubation, filters were exhaustively washed in hybridization buffer at 37 °C and then in 10 mM Tris-HCl *p*H 7.5, 2 mM EDTA at 52 °C. Bound RNA was eluted by boiling for 1 min in 300 μl 10 mM Tris–HCl *p*H 7.5, 2 mM EDTA containing 1.5 μg of *E. coli* tRNA. RNA was precipitated by addition of sodium acetate and ethanol and chilling at −20 °C for 16 h. After centrifugation, precipitated RNA was washed with cold 70% ethanol, dried and redissolved in 1.5 μl 1 mM Tris–HCl *p*H 7.5, 0.1 mM EDTA. For each RNA sample, groups of 30 *Xenopus* oocytes were injected with 50 nl of RNA per egg. Unfractionated LB3 mRNA was at 1 μg ml^{-1} for injection and 50 nl was injected per egg. After incubation of injected oocytes for 4 days, oocyte culture medium was diluted 1:2 in medium containing 5% fetal calf serum, filtered and 5-μl volumes assayed in serial dilutions in 15-μl cultures containing 200 fluorescence-activated cell sorter fractionated 14 day CBA fetal liver progenitor cells[18] or 200 32D C13 cells[20]. Each point is the mean cell count from duplicate cultures after 2 days of incubation.

the mRNA is shown in Fig. 5*a*. The nucleotide sequence given in Fig. 5*b* is a composite derived from both clones. A stretch of 20 adenosine residues, corresponding to the poly(A) tail of the mRNA, preceded by the hexanucleotide AATAAA (found toward the 3′ terminus of most eukaryotic mRNAs[23]) is present at one end of pGM38. This allowed us to orient the sequences of the cDNA clones with that of the mRNA.

The sequence presented in Fig. 5*b* contains a single large open reading frame of 354 nucleotides; the amino acid sequence predicted by this region is presented from the first residue of the mature protein and is given above the nucleotide sequence. From residues 2 to 29, the amino acid sequence is identical (but for four residues) with the partial NH_2-terminal amino acid sequence for GM-CSF (L. Sparrow *et al.*, manuscript in preparation), which is shown above that predicted by the cDNA. Of the four discrepancies, two of the residues (20 and 24) had been only tentatively assigned in the sequence of the protein. The first residue of the mature peptide, which could not be determined in the protein sequence, is predicted by the nucleotide sequence to be isoleucine. The primary structure of the protein deduced from the nucleotide sequence has a molecular weight of 13,500, which is in good agreement with the apparent molecular weight of 16,800 for GM-CSF extensively deglycosylated with endoglycosidase F (A.W.B., unpublished results). Two potential *N*-glycosylation sites (Asn–X–Thr)[24] which occur within the predicted amino acid sequence, are indicated in Fig. 5*b* by asterisks.

pGM37 extends 20 nucleotides 5′ to the first position presented in Fig. 5*b* and does not contain an initiation codon; several hydrophobic amino acids are contained within this region (not shown), a characteristic of signal peptides of secreted proteins[25]. The size of the GM-CSF mRNA is ~1,200 nucleotides (Fig. 2), of which ~150 nucleotides are probably contributed by the poly(A) tail[26]. The 3′ untranslated region is 319 nucleotides and the region encoding the mature protein 354 nucleotides (Fig. 5*b*). Thus some 350 nucleotides remain for the putative NH_2-terminal signal peptide and the 5′ untranslated region.

There are three discrepancies between the nucleotide sequences derived from the two cDNA clones (positions 237, 346 and 507), one of which (position 346) causes an amino acid sequence ambiguity (Gly or Ser at amino acid residue 116). As the mice (C57BL/6) from which the cDNA clones were isolated are highly inbred and hence should be homozygous at the GM-CSF locus, and as there appears to be only one gene encoding GM-CSF in the mouse germ line (see below), it is

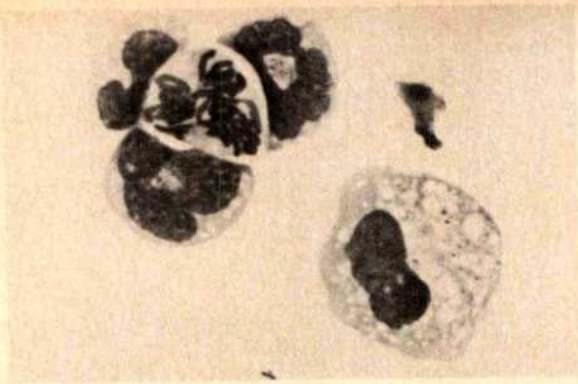


Fig. 4 Four-day suspension culture of purified fetal liver haematopoietic progenitor cells after stimulation by medium from oocytes injected with pGM38-selected mRNA from LB3 cells. Note mitotic activity and the production of maturing granulocytes and macrophages.

likely that these three sequence ambiguities reflect artefacts created during cDNA synthesis[27], and indeed reverse transcriptase has an error frequency on synthetic polynucleotides of approximately one in 600 nucleotides (ref. 28).

GM-CSF is encoded by a unique gene

As there are known to be multiple molecular forms of GM-CSF isolated from various mouse organs[29], we tested how many genes the GM-CSF cDNA could detect in the murine genome. BALB/c embryo or C57BL/6 liver DNA digested with various restriction endonucleases was fractionated on agarose gels, transferred to nitrocellulose[30] and hybridized with a fragment containing the entire cDNA insert in pGM38. In both mouse strains, a single *Eco*RI fragment (Fig. 6, lanes 1, 2), a single *Pst*I fragment (lanes 3, 4) and a single *Bam*HI fragment (not shown) were detected. Since the *Pst*I fragment is only 2.5 kb in length it is unlikely that this fragment could accommodate more than one gene. We therefore conclude that there is only one gene encoding the lung-type GM-CSF in the mouse germ line, barring the unlikely possibility that two (or more) genes are flanked by identically positioned *Eco*RI, *Pst*I and *Bam*HI restriction sites.

Figure 6 also reveals that the GM-CSF gene is contained within the same *Eco*RI fragment in DNA from concanavalin A-stimulated LB3 cells (lane 6) as in embryo DNA (lane 5). No additional bands were evident in LB3 DNA nor was the intensity of hybridization different from that of embryo DNA, from which we infer that there are no alterations in the copy number or gross changes in the context of the GM-CSF gene in cells which synthesize large quantities of GM-CSF.

In order to determine the chromosome on which the GM-CSF gene resides, we examined DNA from seven mouse–Chinese hamster hybrid cell lines which retain various mouse chromosomes (see Table 1 in ref. 31). Southern analysis of the cell lines (not shown) indicated that the 22 kb *Eco*RI fragment present in the murine genome was absent from every hybrid cell line. As chromosome 11 is the only mouse chromosome not retained in any of these lines[31] (a characteristic of mouse–Chinese hamster hybrids[32]), we conclude that the GM-CSF gene is likely to be located on this chromosome. The GM-CSF gene is therefore unlikely to be linked to the four loci in the mouse, *Sl*, *W*, *an* (ref. 33) and *op* (ref. 34), which are involved in the control of haematopoietic stem cell development and which are located on chromosomes 10, 5, 4 (ref. 33) and 12 (ref. 35) respectively.

Comparison with other growth factors

Four distinct colony stimulating factors with distinguishable biochemical and biological properties have been found to act within the granulocyte–macrophage pathway. Furthermore, multiple molecular forms of each of these factors have been observed[16,35–37]: the GM-CSF extracted from various mouse organs have different physical properties[16]; indeed, it has been

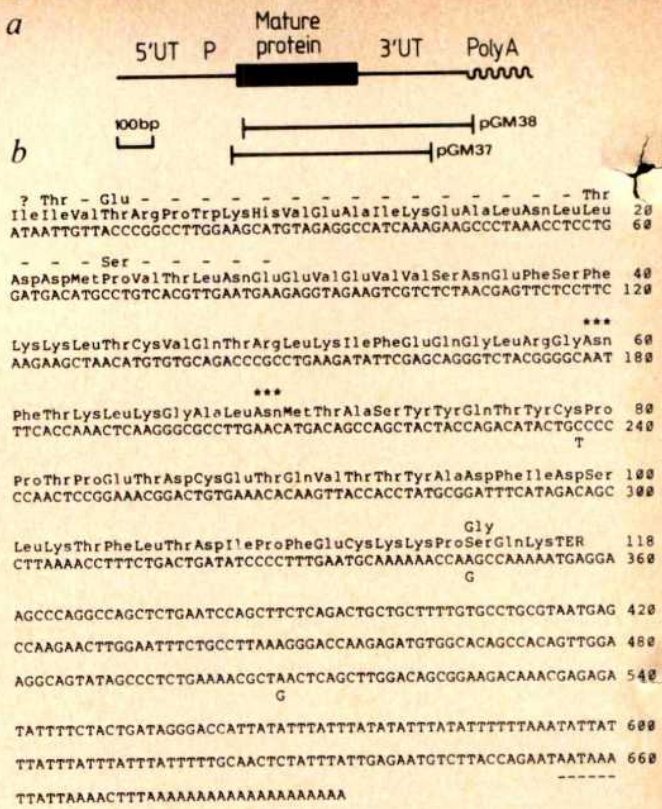


Fig. 5 *a*, Map of the GM-CSF mRNA and of clones pGM37 and pGM38. The mRNA is taken to be 1,200 nucleotides in length. The region of the mRNA encoding the mature protein is shown as a thick line. The untranslated regions are designated by UT and a putative precursor peptide by P. The regions contained within clones pGM37 and pGM38 are indicated with bars. pGM38 extends from nucleotide 14 in the sequence presented in *b* to the poly(A) tail, whereas pGM37 extends from 20 nucleotides 5′ to the sequence presented to position 574. *b*, Nucleotide sequence of GM-CSF mRNA. DNA fragments subcloned in M13 vectors[50] were sequenced by the chain-termination method using dideoxynucleoside trisphosphates[51]. Sequencing reactions were electrophoresed on thermostatically-controlled, 0.2 mm thick, 8% (w:v) polyacrylamide gels[52]. The nucleotide sequence given is a composite of sequence derived from clones pGM37 and pGM38; at three positions where the pGM37 nucleotide sequence differs from that of pGM38 the pGM37 alternatives are given below the line. The sequence of the mRNA-synonymous strand is listed 5′ to 3′, with the predicted amino acid sequence of GM-CSF given above; numbers at the ends of lines indicate the position of the final residue (amino acid or nucleotide) on that line. The partial amino acid sequence determined by L. Sparrow *et al.* (manuscript in preparation) for GM-CSF is indicated above the sequence derived from the clones; at positions where there is no discordance between the two, dashes are given. The first amino acid residue, determined by analysis of the protein, could not be assigned, and is indicated by a question mark. Potential *N*–glycosylation sites[24] are indicated with asterisks, and the putative polyadenylation signal[23] is underlined.

suggested that the GM-CSF produced by T lymphocytes may also represent a distinct form[38]. However, after appropriate treatment to reduce carbohydrate and charge heterogeneity, the GM-CSF from different organs have been shown to have nearly identical biological and biophysical properties[15]. Nonetheless, it is still unclear whether they represent products of multiple related genes or a single gene product with post-translational modifications. However, as we have shown here that there is only one gene in the mouse germ line corresponding to the lung-type GM-CSF, it is highly likely that the differences in the GM-CSF produced by different adult organs reflect post-translational modifications of a single product. Moreover, since the lung-derived GM-CSF cDNA can hybridize to the GM-CSF mRNA in the T-cell line LB3, this T-cell derived GM-CSF must be

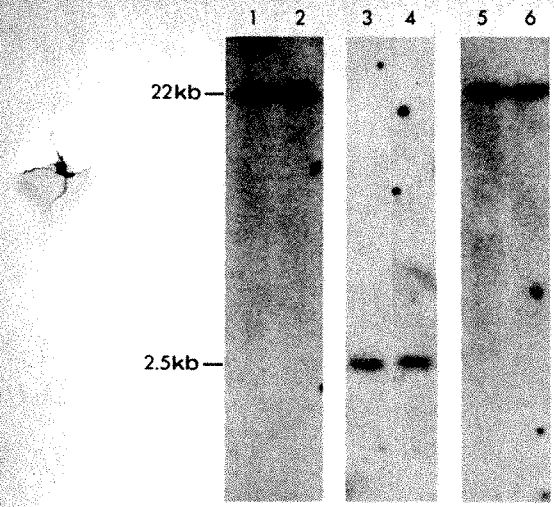


Fig. 6 Detection of the GM-CSF gene. DNA (20 μg) was digested with *Eco*RI (lanes 1, 2, 5, 6) or *Pst*1 (lanes 3, 4) and electrophoresed on 0.8% agarose gels. After transfer to nitrocellulose[29], the filters were hybridized with a fragment spanning the entire cDNA insert of pGM38, ^{32}P-labelled by nick-translation[23]. Hybridization was at 65 °C for 16 h in 2×SSC, 0.1% SDS, 0.2% Ficoll, 0.2% PVP, 0.2% BSA containing 50 μg ml^{-1} of denatured salmon sperm DNA. The DNA used in lanes 1, 3 and 5 was from 10 day old BALB/c mouse embryos, that in lanes 2 and 4 from C57BL/6 livers and that in track 6 from concanavalin A-stimulated LB3 cells (see legend to Fig. 2). Fragment sizes are given in kilobases and were determined[53] by reference to a *Hin*dIII digest of bacteriophage λ DNA.

encoded by the same gene as the lung-type GM-CSF. Variants with different amino acid sequences from that of the lung-type GM-CSF, and whose genes do not cross-hybridize with the lung-type GM-CSF, may of course exist.

It is intriguing to compare the sequence of GM-CSF with that of multi-CSF which, in addition to its other activities, has similar actions to GM-CSF in stimulating the growth of granulocyte and macrophage colonies from committed progenitor cells[11,35]. It might not have been surprising if these two factors had shown primary amino acid sequence similarity, reflecting a common receptor binding site or similar modes of action within the cell. This is, however, not the case. When we compared the amino acid sequence of GM-CSF (Fig. 5*b*) with that for murine multi-CSF[13,14] using the ALIGN computer program[39], we found only one cluster of three consecutive amino acids in common between GM-CSF and multi-CSF: Thr-Leu-Asn at position 26–28 of GM-CSF and 40–42 of multi-CSF. The dissimilarity of these two regulators is emphasized further by the differences in the predicted secondary[40] and tertiary[41] structures. Whereas GM-CSF is predicted to contain two long α-helical segments towards the NH_2-terminus of the molecule (from residues 7–22 and 25–40), the only notable α-helix predicted in multi-CSF is towards the C-terminus (residues 123–132). Furthermore, the hydrophilicity profiles for the two molecules are quite different, suggesting that their surface structures bear little resemblance to one another. Thus it would appear that GM-CSF and multi-CSF represent an example where similar biological activities have arisen by convergent evolution.

The apparent structural dissimilarity of GM-CSF and multi-CSF suggests that they may have different receptor binding sites; indeed, the simplest model would be that these binding sites represent two independent receptors. In this model, a cell responsive to both GM-CSF and multi-CSF would have both receptors, either of which when triggered would stimulate proliferation and differentiation, whereas a cell responsive only to multi-CSF would have a receptor only for this factor. However, this model is complicated by the observation that multi-CSF can compete efficiently with GM-CSF for binding to its receptor on bone marrow cells (F. Walker and A.W.B., unpublished results), suggesting that GM-CSF and multi-CSF may in fact interact with the same receptor.

As the molecular cloning of the murine GM-CSF mRNA sequence should facilitate the production of larger quantities of this regulator molecule than previously available, it should be possible to study in detail the interaction of GM-CSF with its receptor(s) and the signals thus generated. Moreover, by *in vitro* mutagenesis it should be possible to dissect the active site(s) of the molecule and to ask whether the different activities of GM-CSF—survival, proliferation and differentiation of progenitor cells—are determined by the same or different active sites.

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Red component activity in dwarf novae

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SU Ursae Majoris systems are short-period binary dwarf novae characterized by two distinct classes of outbursts: 'normal outbursts' of duration ~2 days and less frequent 'superoutbursts' which last ~12 days. Superoutbursts may be present in longer-period systems but could be classified as wide outbursts[1]. Superoutbursts require enhanced mass transfer rates for an extended period of ~10 days to explain their sustained activity[2,3]. During superoutbursts, 'superhumps' are observed[4–6] which modulate the visible light by ≲40% with a period 3–7% longer than the binary period. Measurement of eclipse depths in the two eclipsing SU UMa systems during superoutburst should show whether or not the superhump light is eclipsed and hence make it possible to infer its location either in the eclipsed disk or in the uneclipsed red component. Study of the available eclipse data reported here shows that the superhump light is not eclipsed, so that the red component is the location of the superhump.

Several models[4,7,8] locate the superhump in the vicinity of the accretion disk. Hence the superhump is at least partially eclipsed at the superhump maximum. Furthermore, models based on eccentric structure of the outer disk have significant difficulties on dynamical grounds[9,10]. The red-component superhump model due originally to Warner[5] and also suggested by Vogt[6] and Bath *et al.*[11] places the superhump on the red star and it would not be eclipsed (Fig. 1).

The eclipse acts as a 'stroboscope', revealing the brightness of the non-eclipsed material at various phases of the superhump light. In models where the superhump light is eclipsed the brightness at mid-eclipse will be unchanged by the passage through superhump maximum. A non-eclipsed superhump would cause the eclipsed light level to rise simultaneously with the out-of-eclipse superhump light[11]. The profile of the non-eclipsed light against superhump phase can be mapped using data from several eclipses.

The data analysed are those of Warner[4], and Krzeminski (see ref. 12). We have examined data for Z Chamaeleontis from three superoutbursts, January 1973, February 1980 and December 1982, all observed by Warner at the SAAO[4,5,13]. Plotting the excess light at mid-eclipse relative to the total light of the system (well away from both eclipses and superhumps), against the superhump phase we obtain Fig. 2*a* (see Table 1). Note that this has been corrected for the effect of the fading of the non-eclipsed material[4]. The feature remaining corresponds to both the shape and amplitude of the superhump in Z Cha, as is shown in the typical light curve in Fig. 2*b*. This result demonstrates conclusively that the superhump is uneclipsed in Z Cha at mid-eclipse.

The only other eclipsing SU UMa star, OY Carinae, was observed in superoutburst in January 1980 by Krzeminski. Vogt[12] measured the eclipse depths in magnitudes (relative to the light just out of eclipse) rather than intensities. The result of his method of analysis is that: (1) for eclipsed superhumps the relative eclipse depths will vary in phase with the total superhump light with a maximum at superhump maximum; (2) non-eclipsed superhumps will cause eclipse depths which will vary out of phase with the total superhump light and show a minimum at superhump maximum. The behaviour observed for OY Car[12] is compatible with case (2), and an uneclipsed origin for the superhump light.

Thus, in both observed eclipsing systems the superhump light is visible at mid-eclipse. This is most naturally accounted for as being due to an active, luminous region on the red component, rotating more slowly than the orbital period. The red component then contributes between 20 and 40% of the optical outburst light in an asymmetric 'superspot' region.

If the superhump light originates in the outer rim of the disk it can still be visible at mid-eclipse (except if located close to the line of centres). This light would still be eclipsed, however, and broaden the eclipse, or produce breaks in the eclipse profiles comparable to those visible in quiescent eclipse profiles[13] (produced in the quiescent case by the orbital hump visible in the light curve of these systems). Because such features are not evident in the superhump eclipse profiles we conclude that the superhump light is entirely uneclipsed.

The observations of Z Cha and OY Car made in superoutburst indicate that the eclipse minima vary from their expected times of minima in a manner dependent on the superhump phase at eclipse[4,12]. When presented as O–C (the difference between observed, O, and calculated, C, times of mid-eclipse) diagrams of phase of eclipse minimum versus superhump phase,

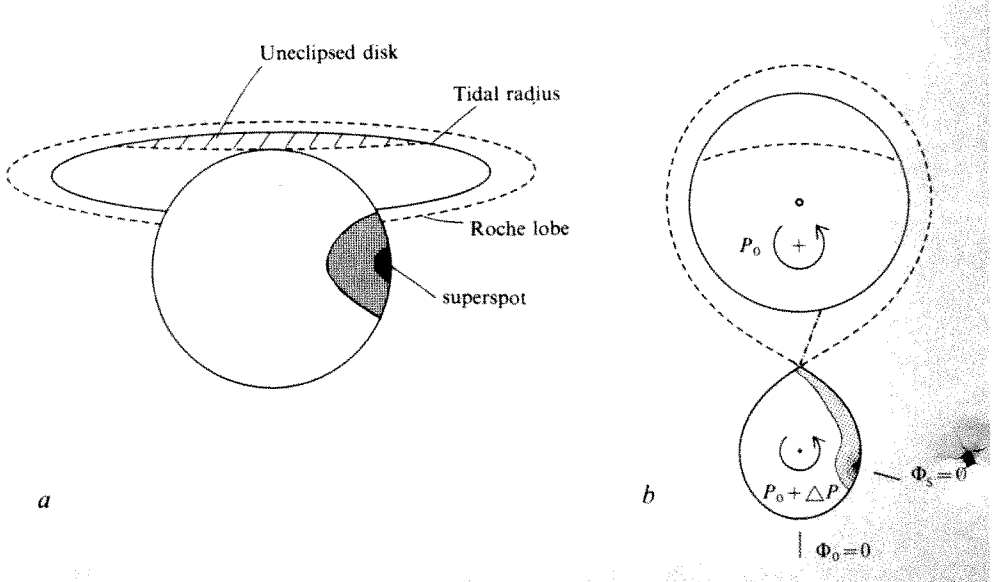


Fig. 1 Structure of the cataclysmic variable Z Chamaeleontis. Parameters used are those due to Vogt[12], and are the most probable for this sytem. *a*, An Earth-bound oberver's view at mid-eclipse, at superhump phase $\phi_s \simeq 0.35$. The extent of the uneclipsed disk surface is shown shaded above the dashed line. Any disk origin for superhumps must be located in this region at all times. A red-component superhump model has no such limitation. The Roche limit is indicated along with the best observational evidence for the disk tidal radius. *b*, The structure present during outburst. Superhump maximum at $\phi_s = 0$ migrates round in orbital phase through non-synchronism, and possibly additional changes in azimuthal structure. When superhump maximum coincides with mid-eclipse the superhump light remains visible.

Table 1 Data from Z Cha during three superoutbursts

Superoutburst	Date	Age of superoutburst (days)	Superhump phase	Relative excess light	Refs
January 1973	8	6.0	−0.32	−0.03	5,13
		6.05	−0.35	−0.02	
		6.1	−0.39	−0.02	
	9	7.1	0.22	0.13	
		7.2	0.15	0.17	
	10	8.1	−0.24	0.06	
	12	9.9	−0.15	0.08	
February 1980	19	2.3	0.48	−0.01	13
		2.35	0.44	0.01	
		2.4	0.40	0.03	
	20	3.5	0.02	0.23	
		3.55	−0.01	0.20	
	23	6.5	−0.40	0.00	
		6.55	−0.44	0.00	
	24	7.6	0.07	0.16	
	25	8.5	−0.43	0.04	
December 1982	14	2.0	−0.48	−0.02	4
		2.05	0.48	0.01	
	16	4.0	−0.43	0.00	
		4.05	−0.46	−0.02	
		4.1	−0.49	0.03	
	17	5.0	0.11	0.22	
		5.05	0.07	0.27	
	19	7.0	0.28	0.15	
		7.05	0.25	0.14	

the times of minima show two effects: an oscillation correlated with superhump phase, and a constant shift. In the data for OY Car, presented in five colours by Vogt[12], both of the above effects are present, and in the case of OY Car find a natural explanation with a red component origin for the superhump light.

A possible explanation of the constant shift has been suggested by Warner[4]. The constant displacement is most naturally due to an asymmetry in the luminosity of the disk due to stream impact and penetration. This displacement of the eclipse minima can be explained by allowing the following lune of the accretion disk to have a luminosity about 5% greater than the preceding lune[4]. If spread over a large region of the surface of the disk, this asymmetrical disk light distribution would not be apparent in the light curve except through its shift of mid-eclipse times.

The red-component model necessarily produces an oscillatory effect. The oscillation is due to the geometrical appearance of the red component. The amplitude of the oscillation is determined by the relative shapes and sizes of the eclipses and superhumps which, in turn are determined by the distribution of light over the red component. As the red component rotates within the binary frame, the 'centre of light' of the faintest region oscillates about the line of centres with the superhump period. A synthetic light curve based on appropriate eclipse and superhump parameters generates the theoretical O–C diagram shown in Fig. 3 which mimics very well the observed behaviour for OY Car[12]. The variation is caused physically by the changing aspect of the red component and its superhump: if the eclipse minimum ($\phi_0 = 0$) lies before superhump maximum ($\phi_5 = 0$) then the asymmetry of the red component advances the eclipse. When the eclipse lies after the superhump then the asymmetry delays the eclipse.

The observed O–C behaviour of Z Cha[4] does not follow the clean positive/negative oscillation seen for OY Car as the superhump passes through eclipse, but rather a positive shift only. No mechanism is apparent within any proposed model which behaves in this fashion. However, complex structure in both the superspot and disk will distort the behaviour shown in Fig. 3, and low superhump luminosity will reduce its amplitude.

In principle, the structure of the superspot region can be probed using the properties of secondary eclipses. Within the present data no secondary eclipse is apparent. This lack of evidence is not, however, inconsistent with a red-component origin for superhump light. In Z Cha the eclipsed area of the

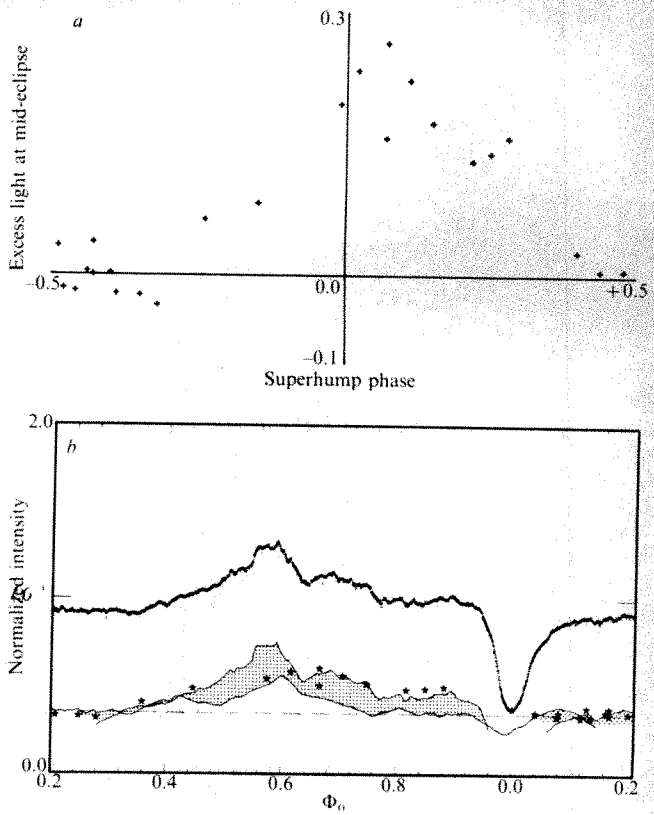


Fig. 2 *a*, The excess light at mid-eclipse in Z Cha as a function of superhump phase. At $\phi_s = 0$ this is typically 25% of the background. The additional light is accounted for by the superhumps, as can be seen in *b* where the points in *a* are replotted under a single cycle of the light curve. The dashed line shows the behaviour of eclipsed superhumps, whereas the shaded region indicates the behaviour of uneclipsed superhumps. The shaded area shows the region enveloped by a bright superhump (early outburst) and a faint superhump (late outburst) in which the eclipse minima will lie, in the case of uneclipsed superhumps. Variations in superhump structure lead to some scatter of the points. Clearly the data show that superhumps are not eclipsed at mid-eclipse.

red component is at most 22% (see Fig. 1). If the superspot is concentrated towards the equatorial belt then the depth of the secondary eclipse is as small as 0.06% of the total light for the case of no temperature change in the eclipsed polar region. Even if the superspot is uniformly distributed over one hemisphere the eclipse light is at most 9% of the total light for bright superhumps, and 5% for more typical cases. Similar constraints apply to OY Car. The present superhump light curves show intrinsic variations (due to superspot structure) at levels comparable to the maximum possible secondary eclipse depth. Such eclipses are therefore not readily visible without more data at times of conjunction of superhump maximum with binary phase = 0.5 to reduce the noise.

In both Z Cha and OY Car the red-component mass is estimated[14] as 0.1–0.2 $M_\odot$ (ref. 14). Such main sequence masses imply quiescent temperatures of ≈4,000 K. Initially the superhump luminosity is ≥90 times brighter than the quiescent luminosity of the red component. With a red-component origin the superhump must then be emitted from a region at a blackbody temperature of at least 12,000 K and probably, with confinement to the equatorial region, superspot temperatures are ≥ 15,000 K. Such a characteristic temperature for the superspot region is in agreement with the colour temperature deduced from UBV fluxes when the superhump is present in both OY Car[12] and the non-eclipsing system VW Hydri[15].

These temperatures are consistent with predicted responses to dynamical, ionization driven overflow instabilities[16,17]. As a result of such instabilities, upwelling of material continues until the ionization zones are brought, by adiabatic expansion, to the

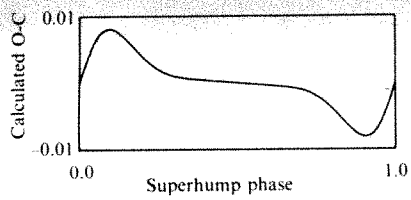


Fig. 3 Model O–C diagram: as the superhump passes through the eclipse, its light alternately shifts the time of minimum to positive, and then negative, phase. The shape and amplitude fit well the observed[12] results for OY Car.

surface. Typical surface temperatures are then 12,000–16,000 K. In normal outbursts these changes will be confined to a region local to the lagrangian point, with typical dimensions of less than a few scale heights ($\leq 0.03R$ in the region of L_1). However, if non-synchronism spreads the instability along the equatorial belt, a significant fraction of the stellar surface can be affected. The streamlines due to non-synchronous rotation will depend on latitude[18], and lead to further stretching of the superspot region along the longitudinal direction. Azimuthal spatial changes in brightness along the equatorial belt would explain the underlying variation in superhump light. The large opacity changes at the expected superhump temperature $>15{,}000$ K may explain the persistent superhump structure on shorter time scales.

The simplest interpretation of the eclipse observations, which has the attraction of being a simple physical structure, is a luminous rotating active region on the equator of the red component. Asynchronous rotation leads to the small period difference between the superhump feature and the binary orbit. Asynchronism may have been driven by the past history of mass and angular momentum exchange (and loss), and gravitational radiation[19]. We stress the close similarity between the marching behaviour of superhumps and the migrating wave in the light curve of RS CVn stars[20], and the geometrical similarity in their suggested structure[21].

The origin of the superspot region is consistent with dynamical instability of the red component at L_1. This powers the superoutburst as fresh unstable material is carried through the L_1 region by slippage of the red component relative to the orbital frame. It remains to be demonstrated that such instabilities will induce disturbances in regions of high latitude. However, pressure gradients will be created over the sub-photospheric equipotential surfaces and may be sufficient to account for the total superhump luminosity by spreading the disturbance to high enough latitudes.

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No IR burst during a type I X-ray burst from the rapid burster (MXB1730−335)

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The rapid X-ray burster, MXB1730−335, discovered by Lewin *et al.*[1] and identified[2] with the compact globular cluster Liller 1, produces thousands of X-ray bursts a day when in an active phase. The X-ray bursts have thermal spectra and can be classified into two types by their spectral evolution during burst decay (see ref. 3 and refs therein). Type I bursts normally occur at intervals of hours or days, their spectra soften during decay and their behaviour can be accounted for well by a thermonuclear flash in accreted material on a neutron star. Type I bursts are produced by all other bursters. Type II bursts are unique to the rapid burster, occur at intervals from a few seconds up to 10 min and their spectra do not soften during burst decay. Type II bursts are probably caused by an instability of the accretion flow onto the neutron star. We report here the absence of an IR burst during a type I X-ray burst from MXB1730−335. The upper limit on the IR luminosity during the X-ray burst is 70 times smaller than those of reported IR bursts from this object. This seems to rule out a connection between type I X-ray bursts and the reported IR bursts.

MXB1730−335 became a controversial object when IR bursts were detected. A total of eight IR bursts have apparently been seen by Kulkarni *et al.*[4] (six bursts with a peak flux of ~5 Jy in the H-band) and Jones *et al.*[5] (two bursts with a peak flux of ~7 Jy in the K-band) observing at different times for a total of 8 h. None of these IR variations corresponds with X-ray bursts of the object as the IR bursts they detected were not accompanied by X-ray observations. On the other hand, on nine occasions, an absence of IR bursts during type II bursts was reported by Sato *et al.*[6] and Glass and Carter (reported by Lawrence *et al.*[3]). The upper limits placed on IR bursts by these authors are estimated to be more than one order of magnitude smaller than those of the reported IR bursts. Clearly, then, type II bursts are not normally accompanied by IR bursts. Kulkarni *et al.*[4] suggested that their IR bursts may be associated with type I bursts. During 1980, Lawrence *et al.*[3] conducted 180 h of IR observations and did not detect a single IR burst. The upper limit during the main part of these observations was <0.2 Jy in the K-band, which is more than one order of magnitude smaller than the reported IR bursts. Unfortunately, the rapid burster was in its quiescent state during these IR observations; the Hakucho satellite, which was looking at this source, detected no X-ray bursts[3].

On 5 August 1983, the X-ray satellite Tenma detected type I bursts almost every 72 min, showing that the rapid burster was in an active phase. On 20 August, type II burst activity was present, and the burster terminated its activity on 31 August[7].

We undertook IR observations on 17 August 1983 using K-band (2.2 μm) photometry on the 1.9-m telescope of Mount Stromlo Observatory. Sky chopping was used in an east–west direction with a beam throw of 96 arc s at 20 Hz. The diameter of the beam was 30 arc s. The time constant on the lock-in amplifier was set at 0.3 s. No IR burst was detected during the observation time of 90 min between 10 h 54 min and 12 h 23 min (UT) on 17 August 1983. During this period, a typical type I burst was detected by Tenma. This burst began at 12 h 13 min 31 s (UT), peaked 8 s after the onset and faded completely into the noise ~80 s after the peak. The peak bolometric X-ray flux was 8×10^{-9} erg s^{-1} cm^{-2}, assuming a black-body spectrum (H. Kunieda *et al.*, personal communication). Figure 1 shows the IR raw data recorded on the strip chart at the time corres-

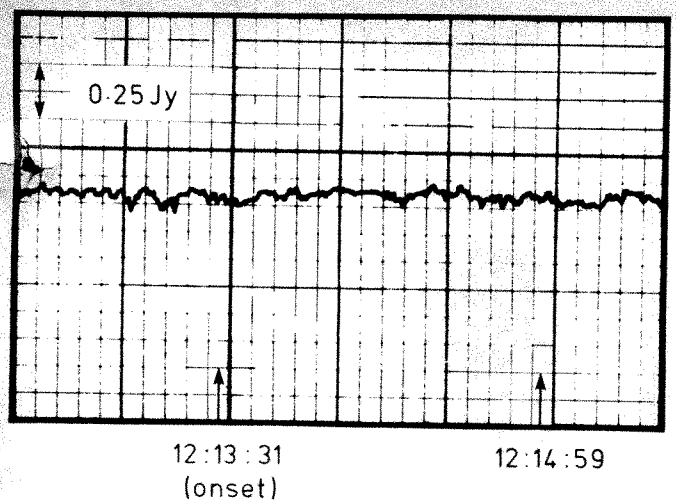


Fig. 1 IR raw data at the K-band corresponding to the time of the X-ray burst, which began at 12 h 13 min 31 s and faded completely into the noise around 12 h 14 min 59 s. The steady level corresponds to the flux from the globular cluster, which has a magnitude of $K = 5.56$ (3.76 Jy) in the 30 arc s aperture.

ponding to the X-ray burst. The steady level corresponds to the flux from the globular cluster. We obtained $K = 5.56$ for the magnitude of the cluster within the 30 arc s aperture, which is consistent with the relation between K-magnitude and aperture size reported by Kleinman *et al.*[8].

The upper limits on variability can be set by using the maximum fluctuations during the observation (times given as UT): $\Delta K < 0.10$ Jy 12 h 13 min 31 s–12 h 14 min 59 s (during the X-ray burst); $\Delta K < 0.25$ Jy 11 h 32 min–12 h 24 min; $\Delta K < 0.4$ Jy 10 h 54 min–11 h 32 min. where 1 Jy corresponds approximately to $K = 7$. Note that the large upper limit of 0.4 Jy in the early part of the observation was probably due to atmospheric conditions and not to luminosity variation of the source.

The upper limit of 0.1 Jy during the type I burst is 70 times smaller than those of reported IR bursts from MXB1730−335. It is unlikely that our time constant of 0.3 s is too long for the detection of IR bursts. Note that Kulkarni *et al.*[4] detected bursts using a time constant of 0.6 s at the H-band as did Jones *et al.*[5] with a time constant of 0.3 s at the K-band.

Our negative result seems to rule out the suggestion by Kulkarni *et al.*[4] that their IR bursts are connected with type I bursts. It is useful to compare our observation with those of other bursters. Coincident X-ray and optical bursts were detected from three type I burst sources, namely 4U/MXB1735−44 (refs 9, 10), MXB1837−05 (ref. 11), and 4U/MXB1636−53 (refs 12, 13). Ten simultaneous X-ray and optical bursts have been observed from 4U/MXB1636−53 by Pedersen *et al.*[12] and Lawrence *et al.*[13] who found that the optical bursts were delayed by a few seconds relative to the X-ray bursts and that the optical burst flux is too large to explain the optical emission as the tail of the black-body X-ray spectrum. It has been suggested[12] that the matter in the vicinity of the X-ray burster absorbs some fractions of the X rays, is heated to $T \sim 50{,}000$ K and re-emits the absorbed energy at optical wavelengths.

The 2.2-μm burst flux which accompanies a type I burst from the rapid burster can be estimated on the assumption that type I bursts from the rapid burster are caused by the same physical process as that suggested for 4U/MXB1636−53; the ratio of the optical peak flux at 3,350–5,250 Å to the bolometric X-ray peak flux is 10^{-3}, after correcting for an interstellar extinction of $A_v = 2.5$ (ref. 11) and assuming that the material heated by the X rays emits a black-body spectrum with $T = 50{,}000$ K at optical and IR wavelengths[13]. The bolometric X-ray burst peak flux of 8×10^{-9} erg s^{-1} cm^{-2} measured by Tenma predicts an apparent 2.2-μm peak flux of 3×10^{-14} erg s^{-1} μm^{-1} cm^{-2} corresponding to 5×10^{-5} Jy assuming an interstellar extinction of $A_K = 1.1$ (ref. 8). This is three orders of magnitude smaller than our upper limit of 0.1 Jy during the type I burst. Hence, if a fraction of type I bursts are accompanied by the reported IR bursts, the mechanism should be quite different from that suggested to explain the well known optical bursts of normal bursters. We note that Lewin *et al.*[14] have also reported the absence of an IR burst during a type I X-ray burst from Ser X-1.

Jones *et al.*[5] pointed out that neither type I nor type II bursts fit the reported IR bursts, whose pattern of occurrence differs from those of X-ray bursts. We conclude that type I bursts do not seem to be accompanied by the reported IR bursts. However, we cannot rule out the possibility that a fraction of type I bursts may be accompanied by the reported IR bursts, as we have only observed one type I burst which shows no IR counterpart.

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First optical detection of atomic deuterium in the upper atmosphere from Spacelab 1

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Measurements of the deuterium-to-hydrogen ratios (D/H) in the fields of meteorology[1,2], tropospheric[3,4] and stratospheric chemistry[5,6], planetology[7,8] and cosmogony[8,9] have previously been performed on molecules in which one hydrogen atom is replaced by one deuterium atom. We report here the first detection of isolated deuterium atoms in the Earth's upper atmosphere through their resonantly scattered Lyman-α emission. An atmospheric deuterium emission of 330 R (1 R = 10^6 photons cm^{-2} s^{-1}) is observed for a tangent altitude of 110 km during Spacelab 1 mission launched on 28 November 1983. The (D/H) ratio of 3×10^{-4} is slightly enriched over the seawater value of 1.6×10^{-4}.

In the terrestrial upper atmosphere, hydrogen atoms are illuminated by the intense solar Lyα line at the resonance wavelength of 121.566 nm in the far UV. The solar Lyα is so wide that it can also illuminate deuterium atoms at the resonance wavelength of 121.533 nm. Through resonant scattering, hydrogen and deuterium can re-emit their Lyα line. Although hydrogen Lyα emission has been observed many times from space, deuterium Lyα has not been detected for two reasons. First, the (D/H) ratio in seawater is only 1.6×10^{-4}. Second, the spectral distance between the two Lyα lines is quite small.

However, the resonance lines of both isotopes are well separated, as their spectral distance of 0.033 nm is much larger than their thermal width of ~0.002 nm. Consequently, the spectral

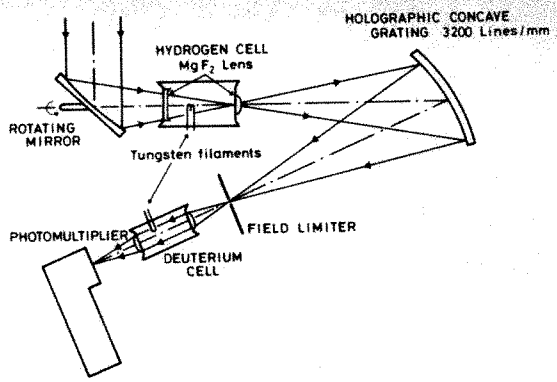


Fig. 1 Optical diagram of experiment 1ES017. The mass of the instrument is 12.5 kg. The dimensions are 685 × 300 × 320 mm and the average power required is 30 W.

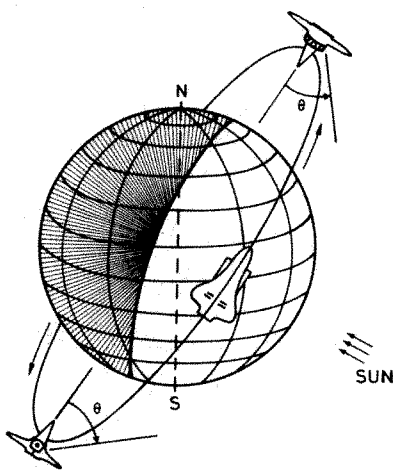


Fig. 2 Geometrical configuration for bright limb observations from an altitude of 250 km. The orbit altitude sketch is greatly exaggerated in respect to the Earth's radius.

resolution needed to distinguish them would have meant using a prohibitively large and heavy classical spectrometer on this first Spacelab mission. We have, therefore, designed and built a lightweight spectrophotometer (Fig. 1) equipped with molecular hydrogen and deuterium absorption cells to suppress selectively one or two of the natural emissions of both isotopes H and D recorded by the instrument. Atmospheric light is collected with a small parabolic off-axis mirror, rotating around one axis to provide a vertical scan of the emission around the limb. The light goes through the hydrogen cell, which is transparent to Lyα when it is not activated, and enters a spectrometer with a single concave holographic grating. A hole at the exit defines both the 2.5° circular field of view of the instrument and a spectral bandwidth of ~4.5 nm centred at Lyα. Thus, the strong atmospheric emission line of atomic oxygen at 130.4 nm is eliminated. The light is then focused on the photomultiplier window through the deuterium cell equipped with MgF_2 lenses. Photons are counted in two counters, sequentially opened in phase with a 5-Hz modulation of the deuterium cell: one counter C_1 is devoted to photons when the deuterium cell is off, the other counter C_2 is devoted to photons when the deuterium cell is activated, that is, when molecular deuterium is dissociated into atoms in the cell.

Fortunately, the atomic hydrogen Lyα emission is saturated, when looking towards the limb, because of the large H column density in the line-of-sight, whereas the optically thin emission I_D of D atoms is proportional to the D column density. As a result, the intensity ratio I_D/I_H is much larger than the (D/H) ratio.

The geometry of observations is illustrated in Fig. 2. The limb is observed from an altitude of 250 km at right angles to the

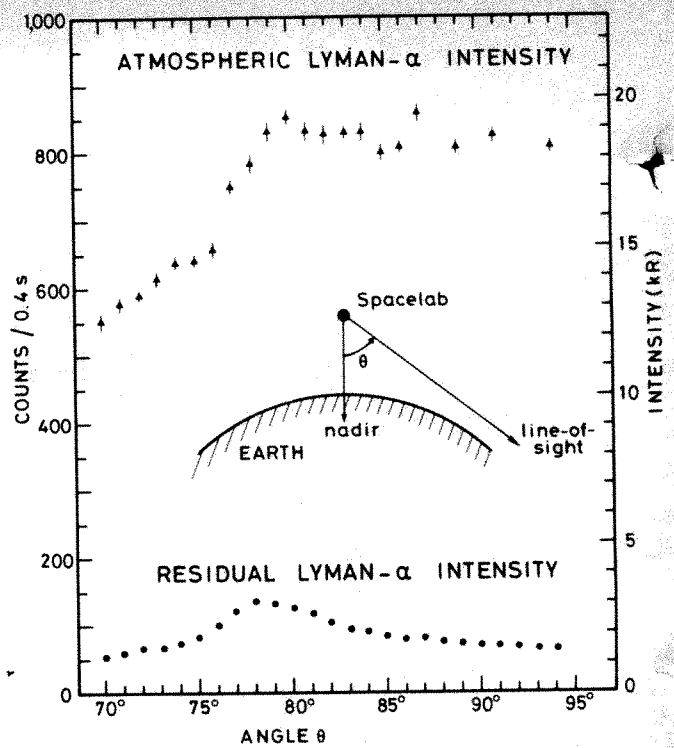


Fig. 3 Measured atmospheric Lyα intensity is shown in the upper part. The residual Lyα intensity indicated in the lower part corresponds to the intensity which is not absorbed when the hydrogen cell is turned on. The left-hand ordinate corresponds to the photomultiplier count rate which is converted into kR in the right-hand scale. The geometrical significance of the observational angle θ is shown in the centre.

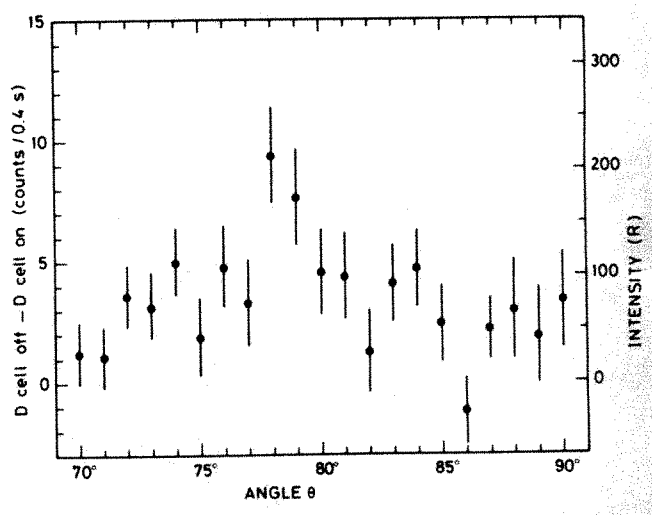


Fig. 4 Atmospheric deuterium Lyα intensity absorbed by the activated deuterium cell. During these measurements the hydrogen cell is permanently on whereas the deuterium cell is alternatively switched on and off. The measured points represent an average of 74 angle scans. Angle θ has the same significance as in Fig. 3.

orbital plane, in order to cancel the Doppler shift associated with the 8 km s^{-1} space shuttle velocity. Otherwise, the absorption cells would have had little or no effect on the atmospheric H and D emissions.

The measured intensity, which essentially results from H Lyα emission, is displayed in the upper part of Fig. 3 as a function of the angle θ with the local vertical. When approaching the limb from below, the H Lyα increases up to the point where the line-of-sight passes above 110 km altitude, corresponding to $\theta = 78°$. Above this height, absorption of Lyα emission by atmospheric molecular oxygen becomes negligible and the Lyα intensity reaches 20 kR.

When a tungsten ribbon is heated electrically in the H_2 cell, an optical thickness $\tau_H \cong 600$ of atomic H is created inside the cell, which absorbs a large fraction (80–90%) of the total signal. The H_2 cell acts as a 'negative' filter and lets through every photon with a wavelength lying outside the absorption profile, which has an equivalent width of 0.006 nm. The transmitted signal is recorded by counter C_1 and shown at the bottom of Fig. 3. It contains mainly H Lyα photons from the linewings, which extend slightly outside the negative filter because of saturation effects.

A small contribution of atmospheric deuterium Lyα emission can then be identified by using the deuterium cell, which creates an optical thickness $\tau_D \cong 1.5$, representing a negative filter selective for D Lyα emission. For $\tau_D = 1.5$, it can be shown that ~60% of the deuterium intensity I_D is absorbed in the cell, and the difference between the two counters $C_1 - C_2$ represents a measurement of $0.6 I_D$. This absorbed intensity is plotted both in counts per 0.4 s and in Rayleighs in Fig. 4.

Seventy-four scans around the limb were averaged and binned in steps of $\Delta\theta = 1°$ to obtain the curve in Fig. 4, representing ~100 min of measurements spread over three orbits and a large range of latitudes. The size of the error bar attached to $C_1 - C_2$ is 2.5 times the error bar attached to counts C_1, which is $(C_1/n)^{1/2}$. Without measurable deuterium emission I_D, the points would be equally positive or negative, which is not the case. Except for one value at $\theta = 86°$, all points are positive, which means that atmospheric deuterium Lyα emission has been unambiguously detected.

A narrow maximum of deuterium emission is found around $\theta = 78°$, at an altitude of ~110 km. This limb brightening effect is expected from the optically thin deuterium emission, when the line-of-sight passes just above the Lyα limb created by atmospheric O_2 absorption. As the field of view of the instrument is 2.5°, it means that the observable deuterium layer is even smaller than what is shown in Fig. 4. However, we can produce a preliminary estimate of the deuterium quantity corresponding to our observations. Because the emitted deuterium Lyα intensity I_D is 330 R, the column number density N_D and the intensity I_D are linked by:

$$I_D(\mathrm{R}) = 10^{-6}\sigma F_s N_D = 10^{-6}\, g\, N_D$$

where F_s is the solar flux[10] of 2.8×10^{12} photons cm^{-2} s^{-1} nm^{-1}. Because the integrated cross-section of D atoms is $\sigma = 5.43 \times 10^{-16}$ cm^2 nm, the excitation rate of D atoms by solar photons is $g = \sigma F_s = 1.52 \times 10^{-3}$ s^{-1}, and these photons are scattered isotropically.

For $I_D = 330$ R, $N_D = 2.2 \times 10^{11}$ atoms cm^{-2} along the line-of-sight tangent at 110-km altitude. We have compared this integrated density to a vertical distribution model depending mainly on one parameter, the eddy diffusion coefficient K, which indicates roughly the altitude of the homopause level, where the atmosphere goes from a well mixed regime to one in which all gases are distributed according to their own scale height and mass. It has been shown[11] that the (D/H) ratio is sensitive to this value of K and also to the exospheric temperature T. Our value of $N_D = 2.2 \times 10^{11}$ atoms cm^{-2} corresponds to a model with a thermopause temperature of 900 K in which the number density is 2.5×10^3 cm^{-3} at 110 km of altitude, and the (D/H) ratio is of the order of 3×10^{-4}. This modest enrichment by a factor of 2 corresponds to a height-independent eddy diffusion coefficient of 1.3×10^6 cm^2 s^{-1}.

Our deuterium observations were hampered during this Spacelab mission by two problems. First, the sensitivity of our first flight model was a factor of 4 below its nominal value, while a second unit unfortunately achieved nominal sensitivity too late to be accommodated in the Spacelab payload. Second, the orbit, because of date and time of launch, soon shifted towards a position near the terminator, where excitation of deuterium atoms by solar Lyα photons is decreased by a larger O_2 column density. As a result, we could observe deuterium emission only during the first 24 h, although the mission lasted for 10 days. A second mission could help to establish a variation of the homopause level with latitude, a major atmospheric objective.

Nevertheless, we have demonstrated that the natural Lyα emission of deuterium atoms can be used to evaluate their number density in the Earth's atmosphere by a remote-sensing technique.

Finally, this remote-sensing technique could be used with the Space Telescope or space probes for the other planets, in particular for Venus and Mars, where the (D/H) ratio is unknown. For Venus, a (D/H) ratio of 1.6×10^{-2} was found[7] in a cloud droplet sucked by a neutral mass spectrometer on board the large probe of Pioneer Venus mission. Such a high value can be explained only by a fantastic differential escape of both isotopes[12,13], the heavier D atoms being left behind in the atmosphere of Venus. The quantity of water at the origin of Venus was at least 100 times larger[7] than it is now.

Such an important clue about the history of water on Venus deserves further investigation. We have demonstrated the feasibility of the deuterium Lyα detection technique. It can now be applied to detailed terrestrial and planetary studies.

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Crustal evolution in north-east and east Africa from model Nd ages

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The relationships between the various crustal provinces that comprise north-east and east Africa are far from clear and reflect diverse tectonic and magmatic processes which took place during the Proterozoic. Here we present the results of an Nd isotope study on the major rock units of the Pan-African (1,100–500 Myr BP) terrane. Charnockites from Jabel Uweinat, a basement inlier at the junction of Egypt, Libya and the Sudan, yield middle Archaean model Nd ages, whilst model ages of <1,200 Myr have been obtained in a belt from the Eastern Desert of Egypt to north-west Kenya. This, therefore, represents a westward extension of the juvenile Pan-African crust of the Arabian Shield (Fig. 1). The shield itself is characterized by comparatively rapid crustal growth[1], probably in an accreting island arc environment[2]. Between the two areas, there is a marginal zone of Pan-African rocks with relatively low $\varepsilon_{Nd}(T)$ values (see Table 1) due to increased contributions from the pre-Pan African crust. Overall, the Pan-African rocks from north-east and east Africa and those from the Damara of Namibia[3] exhibit a wide range of $\varepsilon_{Nd}(T)$ from +7.5 to −18.0 which reflects regional changes in tectonic style and is not readily reconciled with simple models for the evolution of average continental crust.

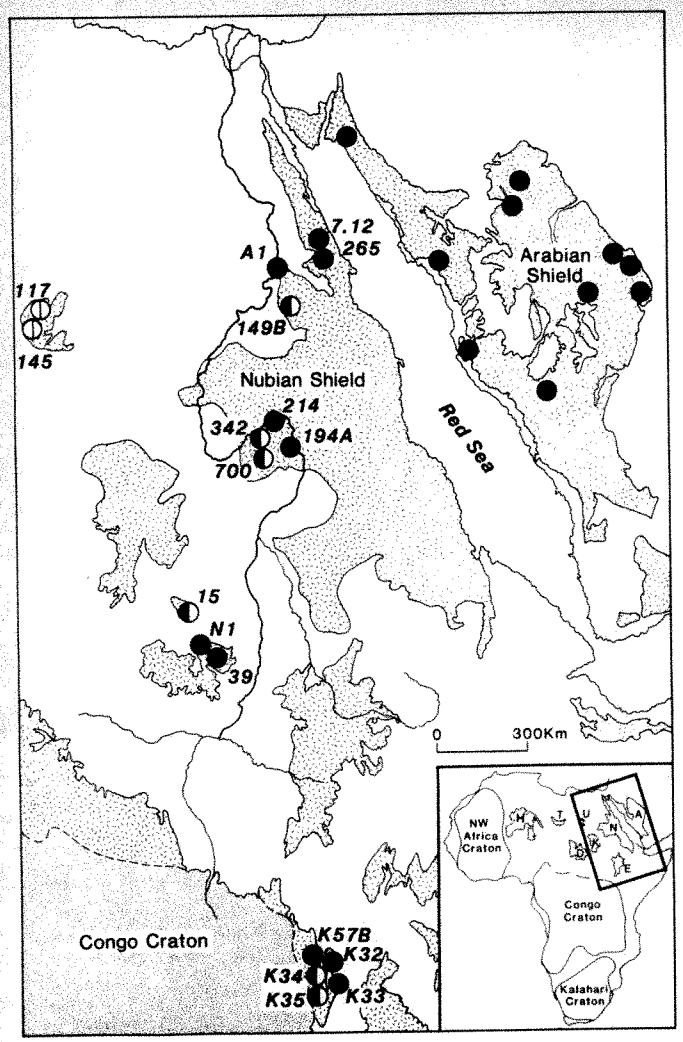


Fig. 1 Sample location map showing extent of basement outcrop and distribution of Nd model ages in north-east and east Africa and Arabia. ●, 800–1,300 Myr; ◐, 1,600–1,900 Myr; ⊙, 3,000–3,200 Myr. Inset shows the location of the study area relative to African cratons of Archaean age, and basement inliers at Hoggar (H), Tibesti (T), Jabel Uweinat (U), Darfur (D), Kordofan (K) and western Ethiopia (E); also shown are Nubian (N) and Arabian (A) shields.

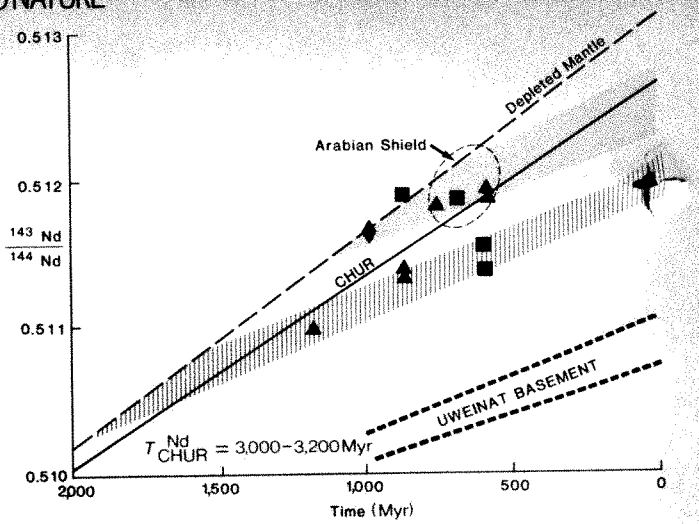


Fig. 2 Variations in initial $^{143}Nd/^{144}Nd$ ratios in samples from north-east and east Africa. The stippled region encloses the evolution lines for samples 265, 7.12, A1, 194A, 214, N1, 39, K32, K33 and K57B. The hatched region encloses the evolution lines for samples 342, 700, 15, 149B, K34 and K35. Evolution paths truncated by inferred light REE-depleted mantle[6]. ■, Granitic and meta-igneous samples; ▲, metasediments; ◆, amphibolites. Field of Arabian data taken from ref. 1.

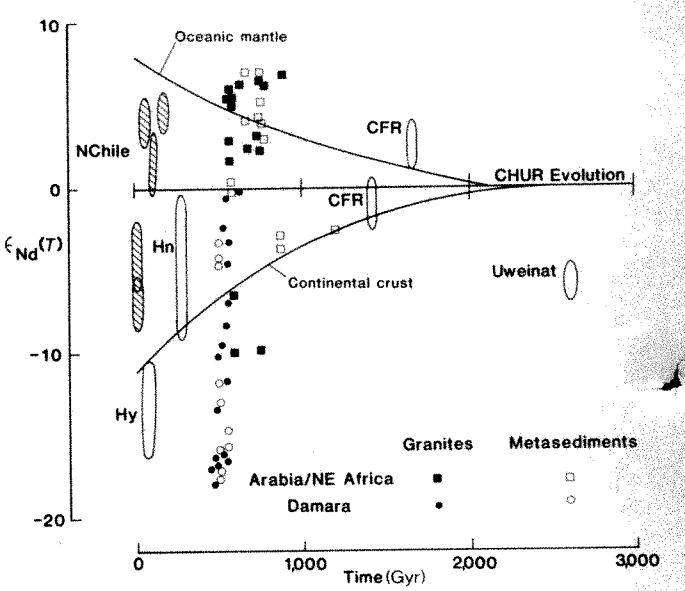


Fig. 3 Mixing model curves for evolution of oceanic mantle and continental crust assuming that the last two orogenies contributed to continental crust[27]. Pan-African data are taken from north-east Africa, Arabia[1] and Damara[3,30]. Also shown are data from northern Chile[29,31], Himalayas (Hy)[27], the Hercynian of south-west Europe (Hn)[27,28] and the Columbia Front Range (CFR)[6].

The Karkur Murr charnockites from Jabel Uweinat (Fig. 1) form the oldest known basement in north-east Africa[4,5] and two samples have model Nd ages, relative to depleted mantle[6], of 3,000 and 3,200 Myr (Fig. 2, Table 1). This confirms their Archaean age, while their low $\varepsilon_{Nd}(T)$ and high $\varepsilon_{Sr}(T)$ values indicate that the event dated at 2,617 ± 21 Myr by Rb/Sr whole rock analyses[5] involved considerable crustal reworking. Figure 2 also illustrates that none of the other samples from this study has a uniquely Archaean source. Most samples from Egypt and the Sudan have Nd model ages between 750 and 1,150 Myr. They are therefore isotopically indistinguishable from the juvenile Pan-African crust of the Arabian Shield[1,7]. The amphibolite from the Marich area (K57B) of north-west Kenya[8,9] and the foliated granodiorite from the Abu Harik complex (194A) of the Bayuda Desert of the western Nubian Shield[10] lie just above the evolution path for average depleted mantle, as do some of the Pan-African rocks from Arabia[1,7]. The metasediment (265) and the foliated granite (7.12) from the Hafafit region of Egypt represent the highest grade of metamorphism in the Eastern Desert[11] and lie well within this age group, indicating that, despite their strong deformation, they are Pan-African in age and were derived from juvenile crust. The Aswan granite (A1) has high initial $\varepsilon_{Nd}(T)$ (+2.3) and low initial $\varepsilon_{Sr}(T)$ (−8.5), values similar to those reported from other younger granites in the Eastern Desert[12] and to many recent mantle-derived rocks. However, this lack of evidence for at least a significant contribution from older crustal material is apparently at variance with published radiogenic lead isotope results[13,14]. This may simply reflect differences between the samples analysed; other Rb–Sr studies[15] have reported initial $\varepsilon_{Sr}(T)$ as high as +70, implying that the Aswan granite was isotopically heterogeneous at the time of emplacement. Alternatively, radiogenic lead could have been introduced by subduction processes, as has been recently demonstrated in the Lesser Antilles[16] where samples with $\varepsilon_{Nd}(T) = +2$ have $^{207}Pb/^{204}Pb =$

Table 1 Sr and Nd data from localities in north-east Africa

Locality	Lithology	Sample	Age (Myr)	Ref.	$^{87}Sr/^{86}Sr$	$^{87}Rb/^{86}Sr$	$\varepsilon_{Sr}(T)$	$^{143}Nd/^{144}Nd$	Sm	Nd	$^{147}Sm/^{144}Nd$	$\varepsilon_{Nd}(T)$	T_{DM} (Myr)
Jabel Uweinat	Charnockite (Karkur Murr)	117	2,617±21	5	0.8037	2.515	+99.6	0.51073±3	2.33	14.2	0.099	−4.4	3,000
	Charnockite (Karkur Murr)	145	2,617±21	5	0.8928	4.915	+74.4	0.51103±2	4.63	23.5	0.119	−5.3	3,200
Eastern Desert	Metasedimentary pebble (Hafafit)	265	—	—	—	—	—	0.51259±2	16.47	84.7	0.118	+6.6*	750
	Metapelite (Wadi Haimur)	149B	1,195	19	—	—	—	0.51200±3	6.80	32.2	0.128	−1.9	1,800
	Foliated granite (Gabal Zabara)	7.12	—	—	—	—	—	0.51255±1	3.43	17.0	0.122	+5.4*	850
	Granite (Aswan)	A1	680±10	13	0.7033‡	—	−8.5	0.51232±1	11.20	68.9	0.098	+2.3	950
Bayuda Desert	Quartzofeldspathic metasediment (Rahaba)	342	874±33	10	—	—	—	0.51206±1	2.56	13.0	0.119	−2.6	1,500
	Quartzofeldspathic metasediment (Rahaba)	700	874±33	10	—	—	—	0.51202±1	2.74	14.0	0.118	−3.3	1,550
	Foliated granodiorite (Abu Harik)	194A	898±51	†	0.7035	0.067	−13.8	0.51277±2	2.18	8.6	0.153	+7.5	750
	Quartzite (Abu Hamed)	214	761±22	†	0.7394	3.356	−12.0	0.51240±2	0.27	1.4	0.117	+3.1	1,050
Central Sudan	Foliated biotite granite (Rashad)	N1	—	—	—	—	—	0.51229±3	4.55	26.9	0.102	+2.2*	1,000
	Foliated granodiorite (Abbasiya)	39	—	—	—	—	—	0.51237±2	10.03	57.4	0.106	+3.5*	950
	Muscovite granite (El Obeid)	15	—	—	—	—	—	0.51214±3	1.99	5.95	0.202	−10.3*	—
North-west Kenya	Granite (Marich)	K34	593±49	†	0.7189	1.382	+45.9	0.51202±2	0.48	2.38	0.122	−6.4	1,650
	Granite (Marich)	K35	593±49	†	0.7127	0.692	+40.7	0.51185±1	0.21	1.03	0.123	−9.9	1,950
	Metapelite (Sekerr)	K32	584±25	†	0.7068	0.310	+3.2	0.51239±2	1.33	6.33	0.127	+0.3	1,100
	Metapelite (Sekerr)	K33	584±25	†	0.7146	1.259	+1.7	0.51237±3	1.09	5.17	0.127	−0.1	1,150
	Amphibolite (Sekerr)	K57B	982±40	†	0.7112	0.589	−8.3	0.51243±3	5.60	28.20	0.120	+5.6	1,000

$\varepsilon_{Nd}(T)$ is the deviation from the value expected in a chondritic reservoir (CHUR) at time T, and is defined as $\varepsilon_{Nd}(T) = 10^4\left[\frac{^{143}Nd/^{144}Nd_{rock}(T)}{^{143}Nd/^{144}Nd_{CHUR}(T)} - 1\right]$ where $^{143}Nd/^{144}Nd_{CHUR}(T) = 0.51264 - 0.1967[(\exp \lambda_{Sm} T) - 1]$. T_{DM} calculated using values from ref. 6.
* Assuming age of 750 Myr. † A.C.R., unpublished data. ‡ Isochron initial $^{87}Sr/^{86}Sr$ ratio.

15.73. Farther south, the quartzite from the Abu Hamed series of the Bayuda Desert[10] (214), and the Rashad granite (N1) and the Abbasiya granodiorite (39) from the Nuba Mountains of southern Kordofan, central Sudan[17] are derived from the source regions of Pan-African age.

The third group of results provides Nd model ages between 1,500 and 2,000 Myr. Model ages in this range were not observed in a recent Nd isotopic study of the Arabian Shield[1] (Fig. 1). In general, such isotopic characteristics may be derived either from a source region of this age or from mixed sources including, presumably, both Archaean and Pan-African material. In the case of the Marich granite (K34, K35), which is exposed 20 km east of the margin of the Congo Craton in north-west Kenya[8,9], its high $\varepsilon_{Sr}(T)$ and low $\varepsilon_{Nd}(T)$ values confirm that it was derived from pre-existing continental crust, and its model age implies that the source included a pre-Pan-African component. The variation in model age between the two samples indicates that isotopic homogeneity was not reached during anatexis, consistent with the scatter of the Rb/Sr whole rock analyses (mean sum of weighted deviates = 51; A.C.R., unpublished data). The Sekerr metasediments (K32, K33) into which the Marich granite is intruded have model ages of 1,150–1,100 Myr, implying that little, if any, of the sediment was derived from the Archaean craton to the west. The muscovite granite (15) from the El Obeid area of Kordofan Province, Central Sudan, also has a low present day $^{143}Nd/^{144}Nd$ ratio relative to that of granite of known Pan-African age, but its model age cannot be calculated since its evolution path closely parallels CHUR. Its flat rare earth element (REE) profile is typical of peraluminous granites and indicates an increase in Sm/Nd during melting. In the simplest model, if it is assumed that melting occurred during the Pan-African from a single source with a Sm/Nd ratio similar to that of average crust, the model Nd age of the source would be at least 2,000 Myr. Thus both the El Obeid and the Marich peraluminous granites contain significant contributions from the pre-Pan-African crust. Similarly, some of the source regions for the Rahaba metasediments in the Bayuda Desert[10] (342, 700), initially mapped as grey gneisses and identified as the oldest Pan-African units in their area[18], and for the amphibolite facies metapelites in southern Egypt[19] (149B) must also predate the major Pan-African crust-forming episodes. These data support the existence of early Proterozoic crust, but argue against the presence of Archaean basement, in the western Nubian Shield and in the Kordofan inlier[20,21].

Nd-isotope studies have therefore identified important differences in the character of the Pan-African terrane across Arabia and north-east Africa. The samples analysed from Arabia all have positive $\varepsilon_{Nd}(T)$ values and none of them is likely to have crustal precursors older than 1,200 Myr (ref. 1) (Figs 1, 2). Similar results were obtained on samples along a belt from southern Egypt to north-west Kenya, but to the north-west there is a shift to lower ε_{Nd} and hence older model Nd ages, culminating in the Archaean ages from Jabel Uweinat. Thus an area of predominantly juvenile Pan-African crust is bounded by one in which rocks apparently formed during the Pan-African event nonetheless have model Nd ages of 1,500–2,000 Myr. However, these rocks are (1) metasediments, and (2) peraluminous granites chosen from areas of higher metamorphic grade as probable crustal melts. There is no evidence of a decrease in $\varepsilon_{Nd}(T)$ in subduction-related magmas (such as A1 and 194A) near the margins of the craton, suggesting that the $\varepsilon_{Nd}(T)$ values of these rocks have not been affected by contamination with upper crustal material. Furthermore, this study has failed to identify any Archaean crust to the east of Jabel Uweinat, and since the Egyptian and Sudanese samples are tightly grouped into two discrete suites, the data suggest that the Archaean basement exposed at Jabel Uweinat was rimmed by younger pre-Pan-African crust of 1,500–2,000 Myr. Two upper intercept U–Pb zircon ages from distinct metasediments in the Eastern Desert—a granite pebble from a conglomerate in Wadi Mobarak (1,960 ± 200 Myr)[22] and an arkosic metasediment from Wadi

Nugrus (1,700 ± 40 Myr)[23]—support such a view. Moreover, in the Hoggar region of north-west Africa a widespread 'Eburnean' event of granulite metamorphism at 2,000 ± 100 Myr has been identified[24]; in the Jabel Uweinat inlier crustal anatexis has been recorded at 1,800 ± 40 Myr (ref. 5); zircons from Phanerozoic sandstones in Tunisia indicate an age of 1,750 ± 100 Myr (ref. 25) and in the Iringa region on the edge of the Mozambique Belt in Tanzania isotopic ages of 1,850 ± 100 Myr are common[26]. Thus it is not unreasonable that crust of this age was sampled by sediments in the Eastern Desert and the Bayuda Desert, and by anatectic melts in central Sudan and north-west Kenya.

The Pan-African event represents a critical stage in the evolution of both large-scale tectonic processes and the continental crust. The volume of stable continental crust has increased, probably at varying rates, since ~4,000 Myr. Since the stabilization of such low Sm/Nd, high Rb/Sr crust is held to be primarily responsible for the high Sm/Nd, low Rb/Sr characteristics of most of the upper mantle, the Nd- and Sr-isotope ratios of the average crust and average upper mantle have also diverged with time[27] (see Fig. 3). Successively younger orogenic episodes, and particularly those over the last 1,000 Myr, should therefore have had more opportunity to remobilize crust which was old enough to have developed distinctive isotope ratios.

Figure 3 summarizes data on granitic rocks from Columbia Front Range[6], the Hercynian of Europe[27,28], the Himalayas[27], and on both extrusives and intrusives from a section across the northern Chilean Andes[29]. Igneous rocks older than 2,000 Myr tend to have initial Nd- and Sr-isotope ratios close to those of the bulk Earth[27] (although Jabel Uweinat offers an interesting exception) while the Phanerozoic examples have variable and often lower $\varepsilon_{Nd}(T)$ values. Thus the young granitoids contain a greater contribution from significantly older crust, either because little old crust survived early in Earth's history and/or younger granites were generated by different processes. Also shown in Fig. 3 are analyses of Pan-African granitoids and metasediments from Arabia[1], north-east Africa and the Damara Belt in Namibia[3,30] with calculated curves for the evolution of average continental crust and upper mantle[27]. Superficially the wide range of $\varepsilon_{Nd}(T)$ in the Pan-African is difficult to reconcile with the curve for the evolution of average crust, but that in part depends on the processes responsible for $\varepsilon_{Nd}(T)$ values in orogenic rocks.

The range of $\varepsilon_{Nd}(T)$ in the Pan-African samples reflects differences in the tectonic regime, and in the age of the pre-existing crust. Areas of significant crustal accretion have ε_{Nd} = +2 to +7, and it requires a marked change in thermal regime, with an increase in metamorphic grade usually associated with crustal thickening, to generate predominantly crustal-derived magmas with low $\varepsilon_{Nd}(T)$ (for example, the Damara[30]). In this case $\varepsilon_{Nd}(T)$ values primarily reflect the age of the pre-existing crust. But crustal thickening is typically a late and comparatively short-lived stage in an orogenic cycle, and thus the low $\varepsilon_{Nd}(T)$ granites are often trivial volumetrically. For example, a detailed section across the northern Chilean Andes[29] has shown that from the start of the present orogenic cycle at ~185 Myr until ~20 Myr the intrusive and extrusive rocks had $\varepsilon_{Nd}(T) = +6$ to 0, and it is only over the past 10–15 Myr, since the development of thick crust, that low $\varepsilon_{Nd}(T)$ rocks have been generated[31] (see also Fig. 3). Similarly, in the Himalayas subduction zone related magmatism associated with the closure of Tethys may have occurred since the Upper Triassic, but the low $\varepsilon_{Nd}(T)$ rocks on Fig. 3 are small-volume, post-collisional crustal melts again formed in response to crustal thickening.

Thus, while crust-forming processes tend to generate magmas with $\varepsilon_{Nd}(T) = +7$ to +2 (as in northern Chile and Arabia, Fig. 3), the Nd-isotope composition of low $\varepsilon_{Nd}(T)$ granites is, like that of sediments, primarily an indication of the age of the local pre-existing crust. As demonstrated here for the Pan-African, the $\varepsilon_{Nd}(T)$ values of magmatic rocks can vary dramatically even in broadly contemporaneous orogenic areas, depending on both the age of the basement and the tectonic environment.

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Effects of acidification and complexation from radiolytic reactions on leach rates of SYNROC C and nuclear waste glass

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Previous work[1] has shown that γ-radiation induced enhancement of leach rates from high-level radioactive waste (HLW) solids can be attributed to radiolytic acidification of the aqueous solution and to radiolytic formation of organic complexing species, for example, $(COOH)_2$, HCOOH. Barkatt *et al.*[1] compared the leach resistance of three HLW glasses and SYNROC D (specifically designed for incorporation of US defence wastes[2]) and found that, at low *p*H and with complexing agents, higher alumina contents in the waste form gave large enhancement of leach rates. Specifically, in acid solution, the elemental leach rates for SYNROC D were increased relative to the glasses by factors between 20 and 1,200. The formulation of SYNROC C, designed by Ringwood[2] for incorporation of reprocessed waste from commercial power reactors, was not tested in these conditions. We report here that SYNROC C is considerably more resistant than the standard HLW glass PNL 76–68 to acid attack and to possible complexation reactions between 90 and 150 °C, despite an alumina content in the formulation that is much higher than that for PNL 76–68. The Al content of SYNROC C is much lower than that of SYNROC D (Table 1) and it is largely present in the hollandite phase after hot pressing. No aluminosilicate phases are formed in SYNROC C and, for this reason, the *p*H dependence is much less marked. These results are significant in assessing the effects of changes induced by the ionizing radiation to which the HLW solid and solution will be exposed during geological isolation.

Table 1 Compositions (wt%) of SYNROC and waste glass PNL 76–68

	SYNROC C (batch 160)	SYNROC D (S21S18)	PNL 76–68
TiO_2	56.0*	21.9	3.0
ZrO_2	11.9*	5.8	1.9
Al_2O_3	5.4*	18.9	—
SiO_2	<0.1*	6.7	38.3*
CaO	13.5*	6.6	2.5*
BaO	5.7*	1.2	0.5*
Fe_2O_3	1.1	23.0	10.3
B_2O_3	—	—	9.1*
SrO	0.7*	0.2	0.5*
Cs_2O	0.7*	0.2	1.2*
Na_2O	—	3.2	12.8
Rare earths and others	5.0	12.3	19.9

* Measured values, otherwise nominal compositions are given.

Ringwood has proposed several different formulations of SYNROC to accommodate various types of high-level nuclear waste[2]. The two formulations that have received most attention are SYNROC C and SYNROC D. Commercial and defence wastes differ in composition and the corresponding SYNROCs also vary considerably in mineralogies and properties[3,4]. The compositions, on an oxide basis, of both SYNROCs and the standard HLW glass PNL 76–68, supplied by the Materials Characterization Center (MCC) at Pacific Northwest Laboratory (PNL)[5], are given in Table 1. SYNROC C can immobilize >20 wt% commercial wastes and has four major phases of perovskite ($CaTiO_3$), hollandite ($Ba(Al, Ti^{3+})_2Ti_6O_{16}$), zirconolite ($CaZrTi_2O_7$) and rutile TiO_2 with some minor metal alloy phases[3]. SYNROC D, however, designed to accommodate up to 65% defence wastes, has major phases of hollandite, zirconolite and perovskite but also an aluminosilicate nepheline ($NaAlSiO_4$) and a spinel titanate phase ($(Fe, Ni, Mn)(Al, Fe)_2O_4$-$(Fe, Ni, Mn)_2TiO_4$)[4]. The last two phases normally comprise about two-thirds of SYNROC D on a volume basis. The high alumina content of SYNROC D arises, not from SYNROC D additives, but from the waste itself.

Barkatt *et al.*[1], in examining the effect of γ-radiolysis on HLW glasses and SYNROC D, reported HNO_3, HCOOH and $(COOH)_2$ at concentrations of 78, 46 and 30 μmol dm^{-3} after 3 days irradiation with a ^{137}Cs source (8.6×10^4 rad h^{-1}). Although it is recognized[1] that a very low *p*H is unlikely to be found in a high-level waste repository (owing to buffering by rock minerals), the local effect of acidification near the waste surface as well as the effect of organic complexing agents in solution may be important. We have tested both effects in a comparison of SYNROC C and PNL 76–68.

The SYNROC C used in our experiments was prepared by the standard oxide-route method[6]. This entailed mixing the precursor oxides or carbonates with a simulated waste solution containing a shortened version of the reference US commercial waste PW-4b[6]. The waste loading was 10 wt% on an oxide basis. After calcination at 1,100 °C in an Ar/3% H_2 atmosphere, 2 wt% Ti powder was added and the blended powder hot-pressed at 1,250 °C for 3 h. The test specimens were 10 mm diameter by 2-mm thick discs of SYNROC C and PNL 76–68 prepared according to the method recommended by the MCC[5]. The flat faces of these discs were ground to a 240–300 grit finish before leaching.

Table 2 compares the leach rates of SYNROC C and PNL 76–68 in static test conditions (MCC-1) at 90 °C for 7 days. The leachants were *p*H 1.8, 4.2, 7.0 and 9.0 buffer solutions. The results clearly show that, in all test conditions, the leach resistance of SYNROC C is superior to that of borosilicate glass PNL 76–68. Furthermore, the leach rate of SYNROC C is less susceptible than glass to changes in *p*H. The difference in the leach rate of the major SYNROC matrix elements, titanium and zirconium, compared with that of the major borosilicate glass matrix elements, silicon and boron, is particularly striking. Although the leach rate of Ti from SYNROC C increases by a factor of >250 when the *p*H is lowered from 7 to 1.8, it is still 200 times lower than that of silicon from glass. The method of reporting results as a ratio of leach rates at two different *p*H values, used by Barkatt *et al.*[1], tends to obscure such information.

Campbell *et al.*[7] have measured the leach rates of SYNROC D in deionized water in identical test conditions (MCC-1). Table 3 shows that SYNROC C is clearly more resistant to leaching than SYNROC D and PNL 76–68. The differences in leach rates of the two types of SYNROC are clearly indicated in the results for Al, Ca and Sr. Table 3 also indicates that the leach rate of Si from SYNROC D is quite high. These facts support the contention[1,2] that it is the aluminosilicate phase in SYNROC D, known to exhibit a sharp dependence of dissolution on *p*H, that is leachable. Conversely, our results show that the absence of aluminosilicate phases in SYNROC C lead to much enhanced resistance to leaching.

Table 4 compares leach rates of SYNROC C and PNL 76–68 in more extreme conditions. Hydrothermal leaching at 150 °C in Teflon-lined Parr autoclaves was used to simulate conditions that could occur if shorter storage times were used before disposal. We have used an acidic solution of nitric acid (0.16 mol dm^{-3}), formic acid (0.37 mol dm^{-3}) and oxalic acid (0.01 mol dm^{-3}) in the proportions 2:1:1 by volume to test the effect of organic acid complexation as well as acidification (*p*H was adjusted to 1.5 with concentrated ammonia solution). The discs were leached in 10 cm^3 of the acid mixture for 1 day; the leachant was then replaced and leaching continued for a further 5 days. Table 4 shows the leach rate for the first day and the average for the entire 6 days. After leaching of the glass, a gel layer had formed on the Teflon container walls and a sediment settled on the bottom of the container. These solids were

Table 2 Leach rate (g m^{-2} day^{-1}) of borosilicate glass (PNL 76–68) and SYNROC C (batch 160) as a function of *p*H (MCC-1 test conditions at 90 °C for 7 days)

	Buffer *p*H = 1.8		Buffer *p*H = 4.2		Buffer *p*H = 7.0		Buffer *p*H = 9.0	
	Glass	SYNROC	Glass	SYNROC	Glass	SYNROC	Glass	SYNROC
Final *p*H	1.89	1.80	4.35	4.20	7.2	6.9	9.41	9.0
Element								
Cs	8.8	0.88	0.77	0.41	0.57	0.31	1.9	0.39
Sr	6.7	0.11	0.63	0.015	0.39	0.009	0.30	0.009
Ca	4.4	0.10	0.39	0.032	0.25	0.020	0.16	0.021
Ba		0.094		0.030		0.010		0.019
Al		0.11		0.03		0.03		0.05
Si/Ti*	9.7	0.05	0.93	<0.0002	0.54	<0.0002	1.8	<0.0002
B/Zr*	10.0	0.008	0.84	<0.001	0.68	<0.001	2.4	<0.001
Mass	8.0	0.13	0.51	0.07	0.36	0.03	0.81	0.04

* Glass results are for Si and B; SYNROC results are for Ti and Zr.

Table 3 Leach rates ($g\,m^{-2}\,day^{-1}$) of SYNROC and borosilicate waste glass in deionized water (MCC-1 test conditions at 90 °C for 7 days)

	Glass PNL 76–68	SYNROC C batch 160	SYNROC D* batch S21S18
Final *p*H	9.40	5.3	6.0
Element			
Cs	1.8	0.39	0.74
Sr	0.42	0.011	1.17
Ca	0.16	0.017	0.41
Ba	NA	0.021	0.05
Al	NA	0.03	0.47
Si	1.9	NA	2.29
Ti	NA	<0.0002	<0.0003
B	2.4	NA	NA
Zr	NA	<0.001	<0.001
Mass	0.82	0.01	0.30

NA, Not applicable.
* From ref. 7, Cs was presynthesized in hollandite.

Table 4 Average leach rate ($g\,m^{-2}\,day^{-1}$) of borosilicate glass (PNL 76–68) and SYNROC C (batch 114) in hydrothermal conditions (150 °C) at *p*H 1.5 in acid mixture (nitric, formic, oxalic)

Wasteform	Glass		SYNROC C	
Total leach time (days)	1	6	1	6
Final *p*H	2.5	2.6	2.1	2.2
Element				
Cs	43.2 (72.9)	17.1 (27.1)	13.0	4.8
Sr	45.0 (64.7)	15.9 (22.8)	2.5	1.3
Ca	20.1 (*)	8.1 (*)	2.7	1.4
Ba	NA	NA	0.55	0.23
Si	35.6 (74.0)	15.5 (26.1)	NA	NA
Na	54.4 (*)	18.8 (*)	NA	NA
Mass	80.5	28.4	2.5	0.8

NA, Not applicable. Numbers in parentheses include contributions due to sediments.
* Interference precluded analysis.

dissolved and their considerable contribution to the leach rate was determined as shown by the numbers in parentheses in Table 4. The results show clearly that in these extreme conditions, SYNROC C has superior leach resistance. When compared with results at *p*H 1.8 (Table 1), it is seen that the leach rates of both glass and SYNROC are enhanced by the combination of lower *p*H, higher temperature and organic acids by a factor generally <10.

The leach rates we have reported are based on relatively short-term tests (7 days) and relatively low surface area/solution volume ratios. They therefore represent a comparison of relative reactivity towards acid attack and organic acid complexation in initial leaching. The long-term effects of, for instance, solution saturation, reprecipitation and recrystallization have not been considered but some comments on Cs, the most leachable radwaste element, are relevant. Table 4 shows that, in acidic hydrothermal conditions, the leach rate of glass and SYNROC C decreases with time. Glass leaches more or less congruently, but caesium is the most leachable element from SYNROC C. The relatively high initial leach rate of caesium from SYNROC occurs because a small amount (<5%) is incorporated not in the hollandite phase but in intergranular films[8]. The high initial rate is caused by the leaching of this loosely bound caesium. Preferential leaching during the first few days depletes the near-surface region of the intergranular regions and as the thickness of the leached layer increases, so the rate of dissolution decreases. Solomah and Zumwalt[9] found that at 25 °C the caesium leach rate fell from 0.07 $g\,m^{-2}\,day^{-1}$ after 3 days to 0.0004 $g\,m^{-2}\,day^{-1}$ after 100 days. Similarly, Reeve *et al.*[6] found that the leach rate at 100 °C fell from 0.4 $g\,m^{-2}\,day^{-1}$ after 1 day to 0.002 $g\,m^{-2}\,day^{-1}$ after 70 days. The caesium leach rate can be improved further[6] if the SYNROC precursor is prepared by hydrolysis of titanium and zirconium alkoxides following the SANDIA method[10]. The leach rate of Cs from glass is constant when the leachant replacement rate is high[6]. In static conditions the average leach rate decreases by a factor of about three over the first 100 days[11] but, like SYNROC C, the instantaneous leach rate of Cs after 6 months is not measurable[6,11].

It is clear, then, that acidification and complexation effects resulting from γ-radiolytic reactions may be important in assessing different HLW forms. The incorporation of alumina as an aluminosilicate in crystalline materials or as a network-former in glassy materials could result in a large increase in leachability in irradiated aqueous media. SYNROC C, in which the alumina is incorporated into the hollandite phase, shows a much smaller increase than PNL 76–68 or SYNROC D in leachability in conditions expected in the first phase after disposal.

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Evidence of two temperate episodes in late Pleistocene deposits at Marsworth, UK

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In the British Quaternary, two post-Cromerian interglacials, the Hoxnian and the Ipswichian, are recognized[1]. Evidence of additional interglacials in this interval is widely accepted in the oceanic record of Quaternary events[2], and the possibility that at least one additional interglacial of this age is represented in Britain has been discussed[3–5]. However, in the absence of datable interglacial deposits which are seen to overlie one another, the issue has remained controversial. We describe here deposits at Marsworth, UK (Fig. 1) where there is evidence of two temperate episodes, and of intervening periglacial conditions. Stratigraphical superposition is established beyond any reasonable doubt. The later deposit relates to the temperate woodland stage of the Ipswichian Interglacial. Dating of the earlier temperate material by the $^{230}Th/^{234}U$ disequilibrium method indicates an interglacial episode not previously established in the British Quaternary.

Table 1 Mammal faunas of the Marsworth channels

Upper channel	Elephant (indeterminate)
	Dicerorhinus hemitoechus (Falconer)
	Hippopotamus amphibius Linné
	Megaceros giganteus (Blumenbach)
	Cervus elaphus Linné
	Bison priscus Bojanus
	Arvicola terrestris Linné
	Microtus sp.
Lower channel	*Canis lupus* Linné
	Ursus arctos Linné
	Panthera leo Linné
	Mammuthus primigenius Blumenbach
	Equus ferus Boddaert
	Equus hydruntinus Regalia
	Cervid (indeterminate)
	Bovini (indeterminate)
	Microtus oeconomus (Pallas)

At the base of the succession at A (Fig. 2), a channel up to 35 m wide in the surface of the chalk (lower channel) contains sediments of a shallow stream. The channel-fill includes gravelly sands below, passing up into organic mud. The organic mud is confined to the area indicated in section E–F (Fig. 3), but the gravelly sands have been traced continuously in this section. The organic muds and the gravelly sands are overlain by chalky muds full of small chalk pellets. Lenses of water-laid sand and fine gravel are also present. These largely colluvial deposits, up to 4 m thick, are found within and beyond the lower channel and are overlain by a coarser head of chalk and flint debris. This Coombe Rock, 2–3 m thick and involuted in its upper part, can be traced across the site of the lower channel and widely beyond it.

At B (Fig. 2), a separate channel (upper channel), 8 m wide, was present in the surface of the Coombe Rock, truncating the involutions. It contained finely stratified sandy gravel, and marl. There were several small pond-like depressions containing similar deposits nearby.

The lower channel-fill contains abundant fossil material, including molluscs, ostracods, beetles, vertebrates and pollen and plant macrofossils. Travertine is present in clasts up to 0.8 m long, and bears occasional leaf impressions of temperate woodland species, including *Acer*. It also contains Mollusca of temperate woodland habitats, including *Azeca goodalli*, *Discus rotundatus* and *Clausilia bidentata*. The upper channel contained abundant mammalian bone, but no other fossil material.

In the mud of the lower channel-fill, herbs dominate the pollen and macrofossil flora throughout. Gramineae are best represented in the pollen record, with subsidiary amounts of Cyperaceae and many grassland herbs. The small quantities of tree and shrub pollen consist mainly of *Pinus* with occasional *Picea*, *Abies*, *Betula*, *Quercus*, *Ulmus*, *Alnus*, *Salix*, *Juniperus* and *Ribes*. Aquatic pollen is present (*Potamogeton*, *Hippuris* and *Butomus*) and late in the sequence the spore flora includes *Lycopodium clavatum* and *Selaginella selaginoides*. The Mollusca are predominantly undemanding in their habitat requirements (for example, *Trichia hispida*, *Arianta arbustorum*). In the lower part of the sequence, marsh and aquatic species occur, including *Oxyloma pfeifferi* and *Sphaerium corneum*. In the upper part of the sequence they are replaced by grassland species such as

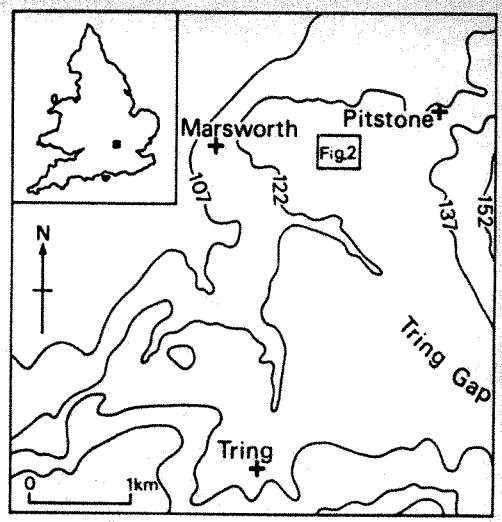


Fig. 1 Map showing the location of Marsworth.

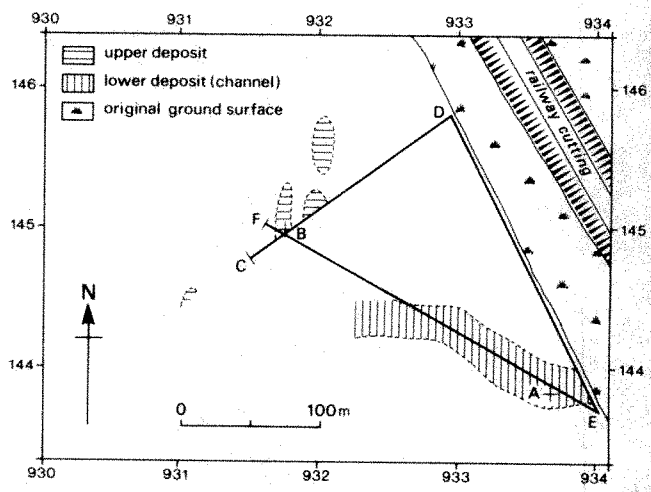


Fig. 2 Pleistocene fossiliferous deposits in Pitstone no. 3 quarry, Marsworth, showing alignment of sections illustrated in Fig. 3. National Grid coordinates are given at the margin (100 km square SP).

Pupilla muscorum, together with many fragments of *A. goodalli* and *C. bidentata*, possibly derived from earlier sediments or soils. The lower part of the sequence yields an ostracod fauna of limited species diversity, dominated by *Prionocypris serrata*, and including *Ilyocypris bradyi*, *Candona neglecta* and *Potamocypris* spp.

Eighty species of Coleoptera are present and suggest a temperate marshy grassland habitat dominated by *Glyceria maxima*, the foodplant of *Donacea semicuprea* and *Notaris acridulus*, which are both common. The abundance of dung beetles suggests that the grassland was grazed, and the small carnivorous beetle *Anotylus gibbulus*, which is also common, is probably associated with the dung of large herbivores. The central and southern European weevil *Stomodes gyrosicollis*, which lives on

Table 2 Uranium series disequilibrium ratios and ages of travertines obtained from the lower channel at Marsworth

Harwell sample no.	Acid-insoluble residue	Uranium content (p.p.m.)	Activity ratios $^{234}U/^{238}U$	Activity ratios $^{230}Th/^{234}U$	$^{230}Th/^{232}Th$	Age (kyr)
HUTH 1359	Nil	0.14 ± 0.02	0.81 ± 0.16	1.910 ± 0.347	5	UD
HUTH 1360	Nil	0.12 ± 0.02	4.41 ± 0.83	1.033 ± 0.107	17	UD
HUTH 1539	Nil	0.06 ± 0.01	2.16 ± 0.19	0.880 ± 0.065	12	171^{+28*}_{-24}
HUTH 2215	Nil	0.08 ± 0.01	1.48 ± 0.13	0.833 ± 0.071	15	165^{+34}_{-27}
HUTH 2237	Nil	0.10 ± 0.01	1.55 ± 0.08	0.795 ± 0.041	37	149^{+16}_{-14}

UD, Undateable. * All errors quoted are $\pm\sigma$ statistical error only.

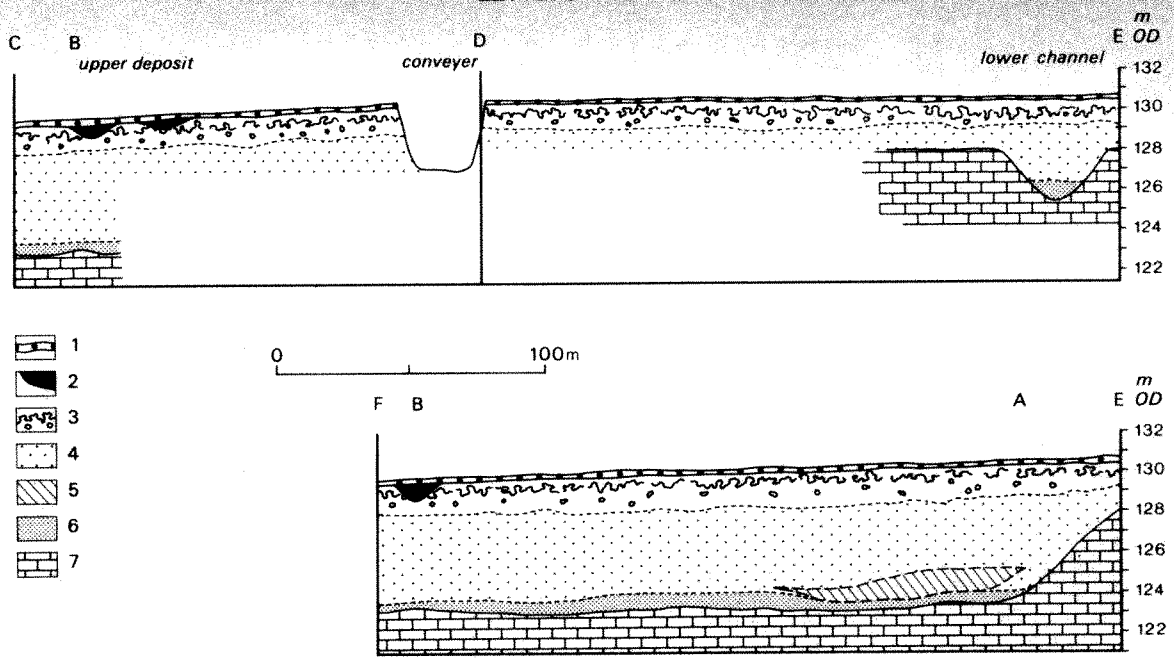


Fig. 3 Sections in Pitstone no. 3 quarry, Marsworth. 1, Topsoil; 2, upper deposit; 3, Coombe Rock with involutions; 4, chalk muds with waterlaid sand and fine gravel; 5, organic muds; 6, fossiliferous gravelly sand; 7, Lower Chalk.

various species of clover, is present. Late in the sequence, an increase of the less thermophilic species, including *Pycnoglypta lurida*, *Arpedium brachypterum* and *Olophrum fuscum*, occurs. The mammal fauna (Table 1) is an assemblage recorded at several sites in Britain[6,7]. These *Mammuthus–Equus* faunas are distinguished by a large form of lion, *Panthera leo*, and a small form of wolf, *Canis lupus*. At Marsworth, the fauna includes *Microtus oeconomus* and the extinct equid, *Equus hydruntinus*.

The fossils in the lower channel-fill indicate a shallow spring-fed stream with backwater slacks, in a local context of marshy grassland. The stream was subsequently replaced by *Carex*-dominated swampy depressions, and these were finally infilled by colluvial debris. The regional vegetation was predominantly grassland throughout the period of record. A climate similar to that of northern England today is represented, with indications in the travertine of earlier warmer conditions, and in the deposits themselves and the fossils contained therein, of slight cooling during sedimentation.

Travertine from the lower channel was dated by the $^{230}Th/^{234}U$ disequilibrium method (Table 2). Samples HUTH 1539, HUTH 2215 and HUTH 2237 yielded analytical results indicating a good standard of reliability[8–10], and together suggest a range of travertine deposition ages between 140 and 170 kyr.

In the upper channel, the mammal fauna (Table 1) is a characteristic temperate woodland assemblage, known from many other British sites and apparently restricted to the Ipswichian Interglacial[6,7,11]. Speleothem enclosing a similar fauna in Yorkshire has yielded uranium series dates around 120 kyr BP (ref. 12). Stratigraphical relationships at Marsworth (Figs 2, 3) show that the upper channel-fill overlies the lower. The coombe deposits separating the channel-fills suggest an intervening period of severe periglacial conditions.

Dating of the travertine in the lower channel indicates a pre-Ipswichian age, but one too young to fall within the Hoxnian. In addition, the mammal fauna in the lower channel is unlike faunas of Hoxnian age. *Mammuthus–Equus* faunas occur in middle Devensian contexts[6,7], but differ in composition from that at Marsworth. Sites having *Mammuthus–Equus* assemblages comparable with Marsworth have generally been assigned to the late Ipswichian[6,7]. The Marsworth evidence demonstrates that a pre-Ipswichian age is possible for such assemblages[13,14].

The beetle *A. gibbulus*, which is common at Marsworth, has not been found in faunas of firmly established Hoxnian or Ipswichian age. It occurs very rarely in middle Devensian deposits, but in assemblages unlike that from Marsworth. It is relatively common (G.R.C., unpublished data) in interglacial sediments at Stoke Goldington in the Great Ouse valley, at Aveley in the Lower Thames Valley, and at Stanton Harcourt in the Upper Thames Valley. At none of these sites are the total fossil assemblages comparable with examples of Hoxnian or Ipswichian age.

The Marsworth pollen shows no clear resemblance to established floras of either interglacial or interstadial status. In the Hoxnian and the Ipswichian, *Picea* was scarce, and while *Abies* was present towards the end of the Hoxnian, it was rare or lacking in the Ipswichian[15]. The middle Devensian, Upton Warren Interstadial has a flora with temperate, boreal and arctic–alpine components, with grassland as the major vegetation[16]. In the early Devensian forested interstadials, *Betula*, *Pinus* and *Picea* are the usual tree components and *Abies* is unknown. At High Lodge, in what may be a Wolstonian interstadial context[15], open coniferous woodland occurs with *Betula*, *Pinus*, *Picea* and *Alnus*, Gramineae and open-ground herbs. Despite the variety of these interstadial floras, they all differ from Marsworth.

We conclude that the travertine in the lower channel represents a previously unrecorded interglacial, intermediate in age between the Hoxnian and the Ipswichian. We note in particular that it preserves temperate woodland species. The sediments of the lower channel, which enclose the travertine, indicate a grassland environment. We note the absence of obligate cold species in the contained fossil assemblages, and conclude that the sediments probably represent conditions succeeding those recorded in the travertine, but within the same temperate episode. It is possible, however, that the sediments denote an additional pre-Ipswichian temperate phase of interstadial status.

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A real-time dynamical forecast of ocean synoptic/mesoscale eddies

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Energetic eddy currents are the oceanic analogue of atmospheric weather and have time scales of days to weeks and space scales of tens to hundreds of kilometres[1]. We have predicted their evolution in time by internal dynamical processes. For the forecasts we transferred initialization data from ship- to shore-based computers, during two 2-week periods in June and July of 1983 from an approximately (150 km)[2] region in the deep north-east Pacific off California. This is, to the best of our knowledge, the first such real-time forecast at sea. The results were substantially successful; the dynamics predicted a prominent zonal jet absent in the initial flow. Also, the model forecast revealed a strong eddy merger event otherwise obscured in the data. Prospects are encouraging for future forecasting, which should have important implications for research and practical operations at sea[2].

The efficient description and prediction of the flow field in the ocean is necessary because of the difficulty and cost of direct observations due both to the opacity and vastness of the sea and to the natural scales of the phenomena. Modern predictive methodology is developing, guided by related experience in meteorology, astronomy and engineering, and based on optimal estimation procedures. Key issues are data assimilation in dynamical models[3], which involves the regular and synoptic gridding (analysis) of gappy multicomponent data sets, and the understanding of initialization and verification procedures. Our concept invokes a descriptive predictive system[4,5] consisting of an observational network, a dynamical model and a statistical model. The statistical model is an anisotropic mixed space-time objective analysis scheme which depends on east, west and time lags[6]; it is used for initialization and verification analyses for dynamical forecasts. The dynamical model is a baroclinic quasigeostrophic model which interrelates the flow, density and pressure fields. The open (water–water) boundary conditions, which allow the utilization of the model in arbitrarily located regions, require the specification of the normal flow everywhere around the region and the vorticity (horizontal gradients of horizontal flow components) on inflow. The model has been documented, calibrated, and applied to hindcasts of the POLYMODE synoptic/dynamics experiment data[5]. With adequate computational resolution and accurate boundary conditions, the model can forecast its interior domain for months, with a normalized r.m.s. error of only a few per cent[7].

Our 'test block' of ocean, located in the California Current System, is centred at 38°15′ N and 126° W, with a mean depth of 4,200 m. Although close to topographic features, the bottom slope is generally only $0(10^{-2})$ or less in the region. Three surveys in 1982 found highly variable flow consisting of current filaments

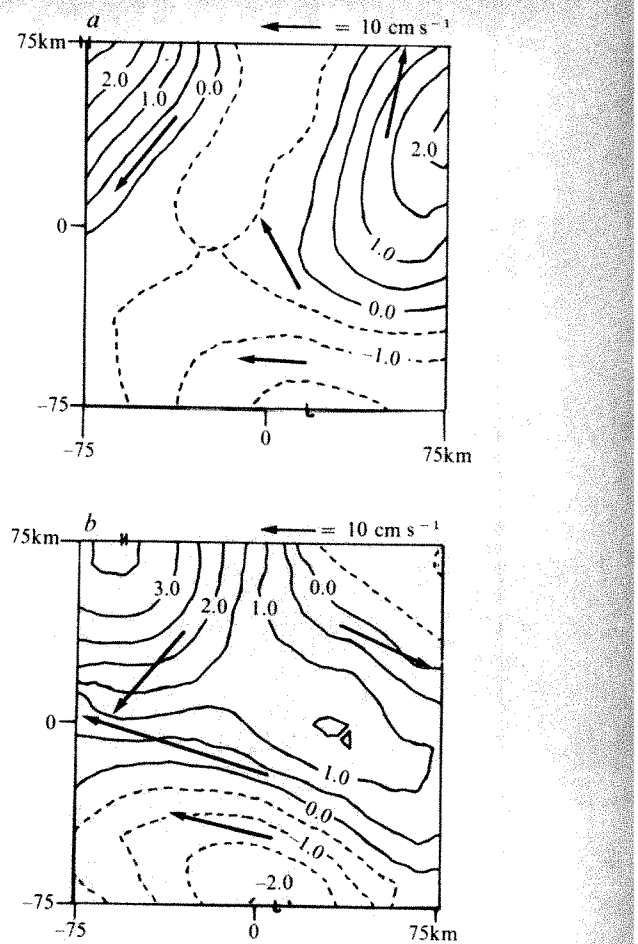


Fig. 1 *a*, Streamfunction (pressure field) at 150-m depth in the main thermocline for Julian day 5506 (20 June 1983), used as the initial condition for forecast and forecast experiment. The centre of the domain is at 38°15′ N, 126° W. The ordinate is rotated 10° west of north. The scale of the streamfunction is 5,000 $m^2 s^{-1}$. Arrows indicate flow speed and direction. The contour interval is 0.5. *b*, As for *a*, Julian day 5520 (4 July 1983) used as verification for both the forecast and forecast experiment, also used in the linear interpolation of boundary conditions for the forecast experiment.

intermingled with cyclonic and anticyclonic eddies. Substantial changes in the flow system can occur within a week[8]. These cruises were carried out to provide the requisite kinematical background for the forecast reported here. In 1983, three hydrographic surveys each of a week duration were obtained 2 weeks apart, to provide two sets of initialization and verification data. Each hydrographic survey consisted of approximately 90 XBT (expendable bathythermograph) measurements of the vertical temperature profile interspersed with 10 CTD (conductivity–temperature–depth) measurements of both the temperature and salinity profiles. Nominal station spacing along the ship track was ~10 km. Temperature and salinity are well correlated in this region so the mean temperature–salinity relationship was used together with the XBT measurements to obtain density profiles and geostrophic currents (dynamic height). Although we plan direct current measurements in future work, the thermocline flow pattern is not sensitive to reasonable assumptions about a geostrophic reference level. Analyses were carried out for each central day by combining data and mapping with a time-independent objective analysis scheme. The first and second analyses are shown in Fig. 1*a*, *b*.

Originally (Fig. 1*a*) the flow in the centre of the region was weak with a strong anticyclonic eddy in the north-east corner, a southwestward current, part of an anticyclonic eddy, in the

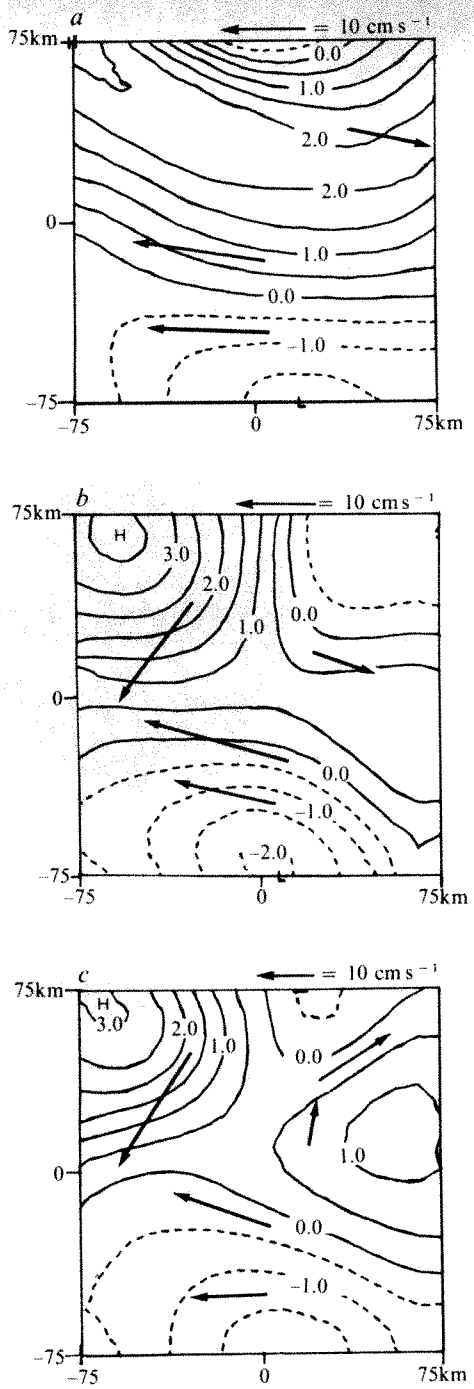


Fig. 2 *a*, Forecast streamfunction at 150-m depth for Julian day 5520 (a 2-week forecast), using persistent boundary conditions (those of Julian day 5506). *b*, Forecast experiment streamfunction at 150 m for Julian day 5520 (a 2-week forecast), using linearly-interpolated boundary conditions (between Julian day 5506 and 5520). *c*, As for *b*, at Julian day 5513 (the forecast after 1 week).

north-west and a segment of cyclonic flow in the southern boundary. Two weeks later (Fig. 1*b*) the flow had dramatically changed; the centre was dominated by a strong zonal jet, the anticylones in the north-west and north-east had apparently merged, and the southern cyclone intensified. After another 2 weeks (not shown), the anticyclonic eddy was further enlarged and the jet, now entering from the eastern edge and oriented towards the south-west, filled the entire central and southern region.

For the dynamical model forecast runs, physical resolution and computational accuracy considerations led to a time step of 3 h, 9 km horizontal resolution and six vertical levels throughout the water column. The hydrographic measurements directly provide only velocity shear in the thermocline, but to initialize the model we need absolute velocity in the thermocline and an estimate of the velocity throughout the water column including the deep water. To estimate the shear below the bottom of the XBT casts, we projected the thermocline flow onto the first baroclinic dynamical mode. The flow in the deep water was then taken to be composed of only the barotropic and first barolinic modes, with an amplitude ratio determined by numerical experiments. Thus determined, the deep flow estimate provides a velocity at 500 m, which was used as a reference value for the thermocline shear. This choice of deep structure, and the ability to estimate the first mode structure from thermocline measurements only, are supported by our related studies with empirical orthogonal functions. After trying different mode ratio assumptions in real time, a barotropic mode was added which had 75% of the energy of the first baroclinic mode and opposed it below the thermocline. This initial mode ratio resulted in the least rapid change of the amplitude ratio of the barotropic to first baroclinic mode at the start of the forecast. The forecasted thermocline flow patterns were in any case found to be insensitive to the assumptions about the mode ratios.

Real-time forecasts were performed by initializing the dynamical model with the analysed data and by simply persisting the initial boundary conditions for the duration of the forecast. Later we performed a dynamical 'forecast experiment' with boundary conditions linearly interpolated in time between the initialization and verification analyses. The forecasts are shown in Fig. 2(*a*, *b*) for the cases of persisted (interpolated) boundary conditions.

During the forecasts the anticyclone in the north-west corner moved into the region, merged with the anticyclone in the north-east corner, which then weakened and disappeared; the small closed contour in the eastern central region of Fig. 1*b* is the remnant of the original north-east corner anticyclone. The southern cyclone enlarged, strengthened and also contributed to the zonal jet. This dynamical behaviour occurred in both the forecast and the forecast experiment and the zonal jet is seen prominently in Figs 1*b*, 2*a*, *b*. The (incorrect) persisted boundary conditions of the forecast alter the flow pattern at the edges and ultimately suppress the north-west corner eddy. However, the forecast experiment with interpolated boundary conditions represents very well the flow pattern and current strengths everywhere. This indicates that the dynamical model can 'project' good resolution boundary data into a rather large domain. Results for the second period were similar.

To illustrate the power of the model as an interpretative physical tool, we show in Fig. 2*c* the flow field a week after the start of the forecast experiment. Without dynamical model interpolation it is impossible to relate unambiguously the synoptic realizations of Fig. 1 separated by only 2 weeks. Figure 2*c* shows the anticyclone originally in the north-east weakened to half its original strength; the jet has formed and the southern cyclone is present. Elucidation of the nonlinear processes inherent in the eddy merger and jet production shown in Fig. 2 will be a contribution to eddy dynamics.

The success of our real-time dynamical forecast is encouraging for the prospects of practical real-time ocean forecasting. The substantial improvement of the dynamical forecast with interpolated boundary condition directs our research now towards: (1) statistical forecasting of the boundary conditions for the dynamical forecast and/or (2) strategies for acquiring new data for boundary condition updating during the forecast. The use of time-dependent objective analysis is essential. The 1983 forecast was a prototype experiment performed as part of the OPTOMA program (Ocean Prediction Through Observation, Modeling and Analysis) which plans a major forecasting experiment in the summer of 1984 when mixed-layer dynamics and satellite imagery will be added to the forecast scheme.

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Light-induced reduction of natural iron(III) oxide and its relevance to phytoplankton

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Primary productivity in natural waters may sometimes be limited by the availability of iron[1–4]. To understand the process it is necessary to know which form(s) of iron can be utilized by phytoplankton. Here we present evidence, from algal culture work and photochemical experiments, which suggests that the prevalent form of iron in most natural waters—particulate iron(III) oxide—is an adequate source of iron for algae, even though iron is taken up only in its soluble, ionic form[5]. This is because the oxide undergoes light-induced reduction, providing a continuous supply of soluble (available) iron(II). Humic substances (HS), co-precipitated with the oxide, participate in the reduction process by reducing the iron(III) directly and/or by maintaining it in a reducible state. These findings suggest that, at least in freshwaters, cessation of phytoplankton growth is rarely due to lack of iron.

The uptake of iron by phytoplankton, and the possibility of iron-limited growth, depend on the chemical speciation of the element[5–7]. It has usually been assumed that algae take up soluble ionic species. However, the stable form of iron in oxic waters of neutral or near-neutral *p*H is Fe(III), which has a low solubility[8]. Therefore, algal growth might cease or be severely curtailed if, after initial growth had used up the small amount of soluble (available) iron, its replenishment was slow in relation to the further demands of the algae[5]. Whether such a situation actually arises depends on the lability of those iron species which, although unavailable *per se*, can be converted into available forms.

In algal culture work, iron is commonly added in a soluble chelated form, such as Fe(III)–EDTA[9]. Such chelates are thought to allow iron to be taken up efficiently through photodecomposition, whereby the chelated iron is reduced and released as soluble Fe(II)[10]. The latter can then be utilized by algae, either directly or after oxidation to ionic Fe(III) forms[5,11].

It has been suggested[5] that the natural chelators for Fe(III) are HS. Although there is no firm evidence for soluble Fe(III) chelates in neutral or alkaline waters, iron oxides adsorb HS[12] and HS co-precipitate with natural iron oxide[13]. Furthermore, at acid *p*H values Fe(III) is readily reduced when HS are present, especially when exposed to light[14]. These facts prompted us to test naturally-occurring iron oxide for light-induced reduction and as an iron source for the common green alga *Monoraphidium contortum* (Thur. in Bréb.) Komárková-Legnerová. We believe this to be the first study of a well-characterized naturally-occurring form in relation to algal iron nutrition.

The natural iron oxide, ~10% by weight HS, accumulates in the hypolimnion of Esthwaite Water in Cumbria, UK, during the summer, being formed through the oxidation of Fe(II) diffusing out of the sediment. The sample used in this work was collected from 1 m above the sediment of Esthwaite Water in July 1983, and was kept at neutral *p*H in the dark at 6 °C before use. Details of material sampled on earlier dates are given in ref. 13.

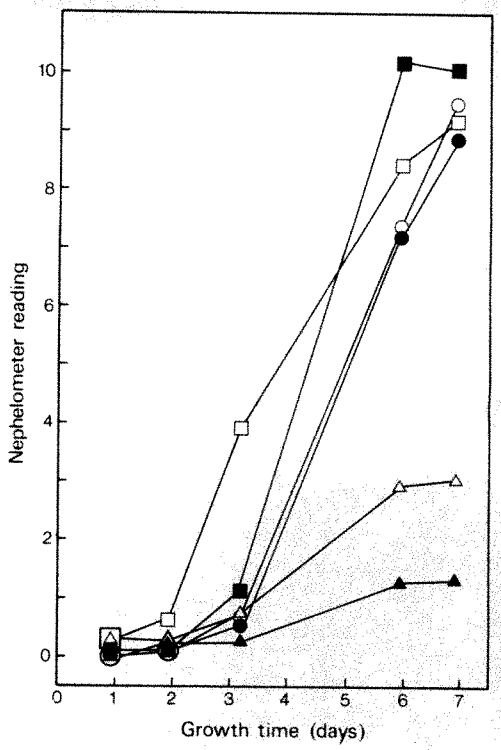


Fig. 1 Growth of *Monoraphidium* on various forms of Fe(III): Esthwaite Water iron oxide (○), synthetic Fe(III)–HS co-precipitate (●), Fe(III)–EDTA (□), fresh ferrihydrite (■), aged ferrihydrite (△), no added iron (▲). The algal growth medium consisted of NaCl, KNO_3, $MgSO_4$ and $CaCl_2$ (all 10^{-3} mol dm^{-3}), KH_2PO_4 (10^{-4} mol dm^{-3}) and Na_2CO_3 (7.5×10^{-4} mol dm^{-3}). When equilibrated with the atmosphere the *p*H was 8.1–8.2. The medium was filtered (0.22 μm Nuclepore) before the addition of iron (2×10^{-6} mol dm^{-3}). Aliquots (10 cm^3) of medium were inoculated with a cultured strain of *Monoraphidium* (L188 from the Freshwater Biological Association Culture Collection; original isolate from Lough Neagh, 1969) at a concentration of $\sim2\times10^7$ cells dm^{-3}. Before inoculation the *Monoraphidium* had been grown to the stationary phase in the same medium with 10^{-6} mol dm^{-3} fresh ferrihydrite as the iron source. The concentration of iron in the experimental tubes due to carry-over in the inoculum was $\sim10^{-8}$ mol dm^{-3}. The inoculated aliquots were contained in transparent polycarbonate tubes and were bubbled continuously with wet air to maintain constant *p*H and P_{CO_2}. The tubes were incubated at 20 °C and illuminated with light (400–700 nm) at 216 μEinstein m^{-2} s^{-1} supplied from below by 4 × 40 W daylight fluorescent tubes. Algal growth was assessed by nephelometry, the scattered light being directly proportional to the concentration of cells in the range 0–3×10^9 cells dm^{-3}: a nephelometer reading of 10 (arbitrary units) corresponds to 1.5×10^9 cells dm^{-3}.

As well as the natural oxide, other forms of Fe(III), were tested. Aged ferrihydrite[15] was prepared by neutralizing $Fe(NO_3)_3$ with NaOH and keeping the precipitate at *p*H 7 for 4 months at 6 °C in the dark. Fresh ferrihydrite was obtained by addition of a solution of $Fe(NO_3)_3$ directly to the culture medium in algal cultures or to the Tris-HCl buffer in the photochemical experiments (see below). A synthetic Fe(III)–humic co-precipitate (HS/Fe = 2, by weight) was prepared by oxidizing $FeSO_4$ in the presence of Penwhirn Reservoir HS[16] and was stored in the same conditions as the aged ferrihydrite. Colloidal haematite (α-Fe_2O_3), goethite (α-FeOOH) and lepidocrocite (γ-FeOOH) were synthesized by methods given in refs 17, 18

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Table 1 Reaction of different forms of Fe(III) with bathophenanthroline disulphonate (BPDS)

Form of Fe(III)	% Fe reacted with BPDS after 16 h	
	Light	Dark
Esthwaite Water iron oxide	6	2
Fe(III)–HS co-precipitate	7	2
Fe(III)–EDTA	34	0
Ferrihydrite Fresh	39	11
Aged	2	1

Iron samples were incubated at a concentration of 2×10^{-4} mol dm^{-3} in 5×10^{-4} mol dm^{-3} BPDS and 0.02 mol dm^{-3} Tris-HCl at *p*H 8.15 and 20 °C. When applied, illumination was as in the algal culture experiments (Fig. 1). The formation of Fe–BPDS complexes was monitored by spectrophotometry at 536 nm. No reduced iron was detectable at zero time.

and 19 respectively. The Fe(III) chelates of EDTA and the siderophore desferrioxamine B[20] were prepared with the chelates in twofold molar excess.

Figure 1 shows that, with a light regime which simulates natural conditions reasonably well, and at a total iron concentration (2×10^{-6} mol dm^{-3}) typical of natural waters, good growth of *Monoraphidium* was obtained with four of the forms of iron studied; Fe(III)–EDTA, the synthetic Fe(III)–HS co-precipitate, the fresh ferrihydrite and the natural Esthwaite Water iron oxide. The aged ferrihydrite was less effective but gave slightly better growth than was obtained with no added iron. Neither the crystalline oxides nor the Fe(III)–desferrioxamine B chelate improved growth.

Table 1 shows the results of photochemical experiments in which the different forms of iron were incubated, either illuminated or in the dark, at the same *p*H (8.15) as the algal cultures, in the presence of bathophenanthroline disulphonate (BPDS). BPDS strongly complexes Fe(II) and so would trap any Fe(II) produced before re-oxidation[5,8,11]. The results for Fe(III)–EDTA are consistent with the well-established photodegradation of the chelate to give Fe(II) (ref. 10). The results for the other four types of iron which enhance algal growth are less clear-cut because in each case iron was to some extent trapped by BPDS in the dark. The dark-iron–BPDS reactions were probably due to the complexation by BPDS of the relatively labile Fe(III), followed by reduction to the BPDS–Fe(II) complex[21] or, when HS were present, due to the reduction of the iron to Fe(II) which then reacted with BPDS. Notwithstanding the reactions in the dark, however, the enhanced production of BPDS-complexed iron when the samples were illuminated indicates that these Fe(III) species each underwent light-induced reduction to give Fe(II) which was subsequently trapped by BPDS. No Fe–BPDS complexation occurred with the crystalline oxides, nor with the Fe(III)–desferrioxamine B chelate.

The results in Table 1 and Fig. 1 show that those forms of Fe (III) which undergo light-induced reduction also support the growth of *Monoraphidium*, in accord with the hypothesis that algae utilize soluble, ionic iron. The Fe(III)–desferrioxamine B chelate is neither reduced nor does it support growth, showing that iron uptake does not depend merely on solubilization.

The results for the humic-free ferrihydrite samples show that HS are not essential for the production of Fe(II). However, the susceptiblity of ferrihydrite to reduction decreases quite rapidly with time, as shown by the results for the fresh and aged samples in Table 1, and also by the observation that only 2 days' ageing at *p*H 8.15 was sufficient to decrease by 80% the amount of Fe trapped by BPDS in 16 h in illuminated conditions. In contrast, the synthetic Fe(III)–humic co-precipitate, in which the iron appears (by transmission electron microscopy) to be present largely as ferrihydrite, was both more readily reduced and better at supporting algal growth than the aged humic-free ferrihydrite, although the two samples were prepared at the same time and stored in the same conditions. The difference might be explained by the co-precipitated HS inhibiting the ageing of the ferrihydrite, or it may be that HS directly reduce aged ferrihydrite under illumination[14].

Irrespective of their mechanism(s) of action, however, we conclude that co-precipitated HS can maintain the susceptibility of iron(III) oxide to light-induced reduction, and that this prolongs the availability to algae of Fe(III). The possible role of HS in making iron available was discussed by Shapiro[6], but in terms of peptization of colloidal particles rather than reduction of Fe(III). The beneficial effects of HS on the growth in culture of a variety of algae[22–25] may well have been due to the HS making iron more susceptible to reduction.

Wells *et al.*[26] considered that algal uptake of iron from synthetic iron(III) (hydr)oxides was related to the rate of (light-independent, non-reductive) dissolution of the solid. However, at neutral or alkaline *p*H, dissolution due to light-induced reduction is likely to be of much greater significance.

The mechanism for making iron available to algae demonstrated by our results is similar to one suggested[27] in relation to manganese uptake by marine phytoplankton, which was based on observations of light-induced reduction by marine HS of synthetic manganese dioxide.

In the epilimnion of Esthwaite Water, much of the particulate iron is present as amorphous iron(III) oxide particles similar in appearance and size to the hypolimnetic material studied above[28]. Assuming that the epilimnetic iron is similarly susceptible to reduction, then at any one time during the summer months there is a large pool of iron (10^{-6}–3×10^{-6} mol dm^{-3}) which can be made available to algae. The pool is continually replenished by iron oxide brought into the lake in inflows or diffusing upwards from the deep water or sediment. It is thus difficult to see how, in this lake, algal uptake could exhaust the supply of iron to the point where further growth was demonstrably iron-limited. Whether this applies to other natural waters remains to be seen. The effects on iron supply of total iron concentration, *p*H and light intensity require investigation, and other natural iron oxides need to be tested for light-induced reduction. Clearly, however, substantiation of suspected iron limitation in nature must take account of the reducible iron oxide pool in relation to the modest iron requirements of planktonic algae.

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A new Lower Devonian plant and the early evolution of leaves

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The fossil record suggests that, by the end of the early Devonian, vascular plants had diversified considerably but they were still relatively simple in their organization[1-3]. Most exhibit much-divided aerial shoots arranged in various patterns but lack clear differentiation into stems and leaves. I describe here a novel plant, discovered in the upper Lower Devonian of Gaspé, Quebec, Canada, whose anatomy is more complex and indicates a level of stem–leaf differentiation significantly more advanced than any known for trimerophytes or other taxa of this age. The presence of a lobed xylem, with mesarch protoxylem elements in major axes, and an elliptically shaped trace in first-order lateral axes in the new plant suggests that by the end of the early Devonian, the evolution of megaphyllous leaves has indeed begun, even though morphologically they were still much divided and non-laminate.

The anatomy of most vascular plants from the Lower Devonian consists of a solid terete or elliptical cylinder of tracheids with centrarch or exarch primary xylem maturation (except in lycophytes or lycophyte precursors with vascularized microphyllous leaves and lobed xylem). A variety of sporangial shapes and modes of attachment (terminal, lateral) occur and some possess unvascularized, usually multicellular emergences. No secondary tissues other than wound tissue are known and reproduction was homosporous or incipiently heterosporous. In contrast, middle and late Devonian plants show the first appearance of more complex stelar patterns, some morphological and/or anatomical distinction between stems and megaphyllous leaves, secondary tissues, arborescence, heterospory and the seed habit. By then, many major plant lineages had become evident, some of which can be linked, at least indirectly, to modern plant groups.

The new plant described here is represented by compression and permineralized specimens collected from the Battery Point Formation near Seal Rock Landing on the north shore of Gaspé Bay, Quebec, Canada. The age of the sediments is determined by dispersed spores which represent McGregor's *caperatus–emsiensis* dispersed spore assemblage zone[4] of early Emsian age. On some compressions, short lengths of axes are permineralized by pyrite or limonite. Some axes also occur in isolated pyrite nodules. So far, only vegetative remains have been found in attachment, although associated fertile organs occur.

The new plant, although similar in external vegetative morphology and complexity to the contemporaneous (Lower Devonian) trimerophyte genera *Pertica*, *Trimerophyton* and, to some extent, *Psilophyton*[5-7], differs significantly on close examination in details of branching pattern (Fig. 1*a*, *b*) as well as anatomy. The major axes, 0.7–1 cm wide, bifurcate into two equal-sized units (Fig. 1*b*, B). Each of these divides equally (isotomously) again or unequally (anisotomously) to produce distantly spaced, spirally arranged lateral branches (Fig. 1*b*, L). The latter divide anisotomously either just above their point of departure or more distally to produce small third-order axes. These divide one to three times and, where complete, terminate in recurved tips. All axes are covered with short (0.3–0.6 mm) hairs which are represented by punctiform scars on axis impressions.

The xylem consists of primary tissues forming a deeply three-lobed protostele (Fig. 1*c*). Three protoxylem areas, one near the end of each arm, are present, and a fourth is often located in the centre (Fig. 1*c*). This type of xylem was observed from sections of the major axes above and below isotomous bifurcations, at the points indicated in Fig. 1*b*.

Xylem maturation thus is mesarch; the protoxylem elements commonly are broken down to form lacunae. Protoxylem areas sometimes appear linked by a band of small cells which probably represent compressed metaxylem elements. The latter exhibit scalariform thickenings or oval and circular pits on all walls (Fig. 1*d*).

Lateral traces are helically arranged. Formation of a lateral trace involves: (1) tangential enlargement of the end of a xylem lobe, first to one side and then the other (Fig. 1*f*, *g*), (2) enlargement and division in protoxylem tissue (Fig. 1*g*, *h*) and (3) separation of outer protoxylem and associated metaxylem elements (Fig. 1*h*, *e*). The tangentially elongate lateral trace usually remains elliptical in shape, with centrally located protoxylem, after leaving the cortex. These traces supply the lateral branches resulting from anisotomies (branches labelled L in Fig. 1*b*).

No early Devonian plant exhibits this particular combination of a lobed vascular strand, mesarch primary xylem maturation and type of lateral trace production. Several groups of middle Devonian plants, namely the Aneurophytales (Progymnospermopsida), Iridopteridales (sensu Stein, 1982) and Cladoxylales[8-13] have variously lobed xylem with protoxylem lacunae surrounded either by remnants of protoxylem or by metaxylem. In these plants, vascular traces to lateral branches usually depart from the lobe tips; at some level the lateral traces differ from the parent axis in size or shape and where different are believed to indicate the presence of leaves. These plants can be distinguished from each other and from the new plant in number and location of protoxylem areas, shape and mode of production of lateral traces, and in the presence (in aneurophytes) of secondary tissues. The new plant might be regarded as a trimerophyte derivative leading towards certain of these younger plant groups, such as the Aneurophytales or Iridopteridales. Other plant groups such as the Cladoxylales and/or ferns probably evolved independently from a trimerophyte ancestor, perhaps at different times.

The anatomy of the new plant offers evidence concerning differentiation of stems leading to the establishment of leaves. In most early Devonian plant groups, evolution of megaphyllous leaves is regarded as being at a very early stage, manifested mainly as variability in branching patterns of stem-like axes. Plants with undoubtedly laminate, megaphyllous leaves occur in the Upper Devonian[14,15], although rare occurrences of incompletely preserved laminate, apparently megaphyllous leaves (*Platyphyllum*) of uncertain affinities have been reported from the Emsian[1,14]. It has been postulated[16,17] that the trimerophytes exhibit early transitions of branch systems to megaphyllous leaves, as follows: (1) in *Psilophyton*, variation in dichotomous to pseudomonopodial branching patterns; (2) in *Pertica* and *Trimerophyton*, the development of a distinct, robust major axis with subordinate lateral branches; and (3) in the latter two genera a tendency for regularly arranged, but pseudomonopodially divided lateral branches. Where anatomy is known, no major change in vascular strand shape occurs in the various orders of branching, except for a slight difference in shape in strands departing to fertile branches in *Psilophyton dawsonii*[16]. The new plant demonstrates a pronounced anatomical change from the lobed xylem of the main axis to an elliptically shaped, first-order lateral branch trace. Comparable changes in vascular strand shape in younger fossil plants, or extant ones, occur between stem and leaf.

The transition from an elliptical centrarch xylem strand, typical of trimerophytes, to a lobed, mesarch one as is present in the new plant and several Middle–Upper Devonian ones of various affinities is here considered possible by loss during development of centrally located protoxylem, leaving separate peripheral strands, and by variable metaxylem development. An intermediate condition may occur in *Psilophyton dawsonii* in those axes from which numerous vegetative branches depart in quick succession and which exhibit a somewhat lobed protoxylem region and irregularly shaped metaxylem. Additionally, a lobed xylem with protoxylem areas in the lobes, as found in the

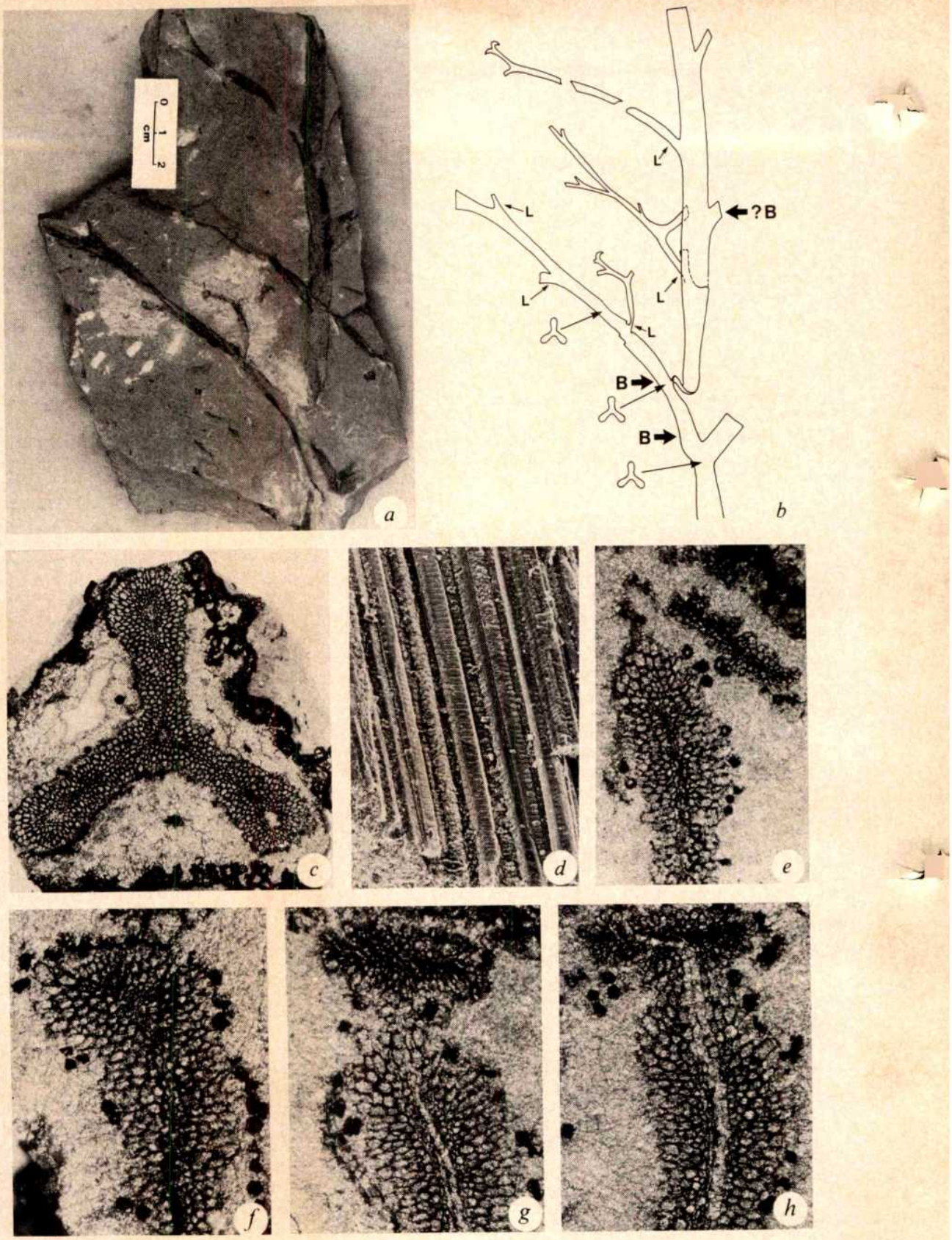


Fig. 1 *a*, Compression/impression showing major components of branching: note two equal bifurcations of the main axis near the base, and production of lateral branches distally. *b*, Line drawing based on tracing photographs of part and counterpart of specimen in *a*. The lateral branch in the middle of the drawing was exposed by splitting of the upper portion of the specimen in *a*. B, Bifurcation of major axis; L, lateral branch; three-lobed symbols indicate regions where permineralized axes were removed. *c*, Transverse section of major axis obtained from specimen in *a*, showing deeply three-lobed stele, protoxylem lacunae and a band of narrow metaxylem cells in the centre. ×20. *d*, Scanning electron micrograph of pyritized tracheids in longitudinal view, showing tapered end walls (lower right) and scalariform to oval pits. *e*, One lobe of a major axis and an elliptical lateral trace; the trace was produced by the sequence in *f*–*h*. ×42. *f*–*h*, Stages in the production of lateral trace. *f*, Xylem at tip of lobe expands to one side. ×56. *g*, Xylem is expanded in both directions; trace is beginning to become detached from lobe. ×55. *h*, Trace further detached from lobe and nearly fully formed; this becomes separate as shown in *e*. ×60.

new plant, may be linked to the very regular pattern of lateral branch departure. In view of this evidence, it is interesting to consider once again what type of xylem existed in plants like the trimerophytes *Pertica* and *Trimerophyton*, which also show a regular pattern of lateral branch departure.

In summary, the new plant, while resembling known Lower Devonian plants in general morphology, is more complex anatomically but can be logically derived from them. Further, it suggests that appendicular structures or leaves were already being differentiated at that time, even though morphologically they still were much divided and non-laminate.

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Recurrent patterns of natural selection in a population of Darwin's finches

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The adaptive significance of morphological traits can be assessed by measuring and identifying the forces of selection acting on them. Boag and Grant[1] documented directional selection in a small population of Darwin's medium ground finches, *Geospiza fortis*, on I. Daphne Major, Galápagos, in 1977. Large beak and body size were favoured at a time of diminishing food supply and high adult mortality. We show here that in two subsequent periods of moderate to high adult mortality (1980 and 1982), the population was subject to the same selection. We have used a recently developed technique[2] to ascertain the targets of direct selection. Beak depth and body weight were commonly under direct selection to increase but, surprisingly, beak width was directly selected to decrease, over all three periods of mortality. The results have implications for our understanding of evolutionary change in morphological traits of Darwin's finches[3–6].

We consider three 18-month periods of adult disappearance, mainly due to mortality[1,3], between June 1976 and December 1982 (Table 1). Each period was associated with a year of low rainfall (Table 1). Less than 5% of adult mortality occurred in the remaining 24 months (January 1978–June 1979 and January–June 1981). Only adults (at least 1 year old) which had been ringed and measured before the periods of high mortality are included in the analyses. Survival of ringed and measured birds was assessed by a detailed census at the end of each period[1,3]. Seed abundance was estimated at this time by counting seeds on plants and in the soil in 50 randomly placed quadrats[1,3]. Four characters (body weight in grams, and beak length, beak depth and beak width in millimetres) have been measured by different observers in a consistent fashion[3,4]. The characters are highly and significantly positively correlated[4–6]. Before each analysis, data were $\log_e$-transformed and then standardized to have zero mean and unit variance.

Table 2 shows the vectors of directional selection differentials (s) and the directional selection gradients (β)[2] associated with mortality in each of the three periods. A selection differential is the difference between the mean value of the character before and after selection, here measured in standard deviation units. The selection gradient comprises the partial regression coefficients of relative fitness (survival) on each character. Relative fitnesses are obtained by dividing absolute fitnesses (0 or 1) by the mean absolute fitness[2], that is, the proportion surviving. Thus, each partial regression coefficient measures intensity of selection acting directly on each character, apart from phenotypic responses due to selection on other correlated and measured characters[2]. After 1978 the ages of birds were known, so we repeated the analysis separately for each age cohort to test for age-specific effects (Table 3).

For all characters and periods, selection differentials are significantly positive, or they are not significantly different from zero: survivors are often larger than those individuals that disappear (Tables 2, 3). The most intense selection in this direction was in 1976–77, when the lowest number of individuals (15%) survived (Table 2). The selection gradients indicate that increases in all measured characters are due almost entirely to selection on beak depth and/or body weight (or to selection on an unmeasured character with which these are highly correlated). Beak length is usually not a target of direct selection, but it increases as a correlated response to selection on beak depth and/or body weight. The partial regressions of relative fitness on beak width are usually negative and often significant: this result was not expected in view of the net increases in the sizes of all traits in 1976–77. Thus, beak shape, as well as size, is subject to selection.

In each of the three periods of mortality, partial regression coefficients of relative fitness on beak depth and body weight are positive and partial regression coefficients of relative fitness on beak width are negative (Tables 2, 3). When the selection over the 1977 drought is divided into three consecutive 6-month periods, this pattern is observed within each period (Table 4). It is also observed when selection on each sex is analysed separately[5].

The repeatedly observed direct selection favouring a small beak width was often intense. It was sufficient to counteract strong selection favouring large beak depth and/or body weight in the 1978 cohort in both 1979–80 and 1981–82, resulting in small selection differentials for all characters, but not in the pre-1976 cohort in 1981–82 (Table 3).

Selection events can be interpreted in terms of feeding conditions. Seed supplies decline during a drought[1], but hard seeds are proportionally common (Table 1). Only the larger individuals can crack these seeds[1,7,8]. Among these large individuals, efficiency at cracking the woody *Tribulus* fruits is a positive function of beak size[7]. Selection for large beak and body size associated with starvation has previously been related to the superior seed handling ability of large individuals[1]. The demonstration here that beak depth is a direct target of selection strengthens this interpretation.

A plausible hypothesis for the selection to decrease beak width is that a relatively narrow beak is favoured in the specialized feeding on *Tribulus* fruits: individuals crack the fruits by placing the side of the beak across a corner and twisting[7]. *Tribulus* is an important food for *G. fortis* during droughts because it remains common (Table 1) and, unlike *Opuntia*, is not defended or consumed by the cactus ground finch, *Geospiza scandens*, the only other common finch on the island. *Tribulus cistoides* may have been introduced to the Galápagos in the past 450 yr[7,9]. If so, the directional selection pressures on beak width that we have detected are relatively new.

This study has shown that selection repeatedly favours individuals with large beak depth and/or body weight in recurring drought conditions. It has also uncovered an unsuspected

Table 1 Selection periods, population sizes before and after each selection period, and seed biomass at the end of drought years (g per 50 m²)

Drought year	Period studied	Rainfall (mm)	Population sizes		Seed abundance		
			Before	After	Small seeds	*Opuntia echios*	*Tribulus cistoides*
1977	June 1976 to December 1977	24.0	1,200	200	1.2	13.6	117.9
1980	June 1979 to December 1980	53.5	260	210	1.7	184.2	130.6
1982	June 1981 to December 1982	51.1	200	150	0.5	21.0	38.6

Seeds are divided into miscellaneous small seeds, and a medium-size seed (*Opuntia*) and a large seed (*Tribulus*), both of which require significant force to crack and cannot be handled by all the finches[1,7,8]. All rain fell in January–May of each period.

Table 2 Standardized selection differentials (s) and standardized selection gradients (β) for periods of mortality

	1976–77		1979–80		1981–82	
	s	$\beta \pm$ s.e.	s	$\beta \pm$ s.e.	s	$\beta \pm$ s.e.
Weight (g)	0.62	0.51 ± 0.14	0.05	0.08 ± 0.06	0.15	0.13 ± 0.08
Beak length (mm)	0.49	0.17 ± 0.18	0.02	−0.04 ± 0.07	0.13	0.06 ± 0.09
Beak depth (mm)	0.60	0.79 ± 0.23	0.03	0.13 ± 0.10	0.12	0.17 ± 0.12
Beak width (mm)	0.49	−0.47 ± 0.21	0.01	−0.14 ± 0.09	0.08	−0.20 ± 0.12
R^2		0.09		0.02		0.06
Sample size		640		192		197
Proportion surviving		0.15		0.78		0.65

All values lie between −1 and 1. Sample sizes refer to birds alive at the beginning of each period. Significance of selection differentials was assessed by t-tests comparing survivors with those who disappeared. Significant R^2 values, partial regression coefficients and selection differentials are underlined ($P < 0.05$).

Table 3 Standardized selection differentials (s) and standardized selection gradients (β) for two cohorts over the 1980 and 1982 drought periods

	1979–80				1981–82			
	Pre-1976		1978		Pre-1976		1978, 1979	
	s	$\beta \pm$ s.e.	s	$\beta \pm$ s.e.	s	$\beta \pm$ s.e.	s	$\beta \pm$ s.e.
Weight (g)	0.03	0.05 ± 0.08	0.11	0.06 ± 0.08	0.24	0.07 ± 0.12	0.08	0.15 ± 0.09
Beak length (mm)	−0.10	−0.20 ± 0.09	0.11	0.08 ± 0.09	0.28	0.16 ± 0.14	0.02	0.01 ± 0.11
Beak depth (mm)	−0.05	0.03 ± 0.12	0.12	0.37 ± 0.15	0.27	0.16 ± 0.19	0.01	0.16 ± 0.15
Beak width (mm)	−0.04	0.05 ± 0.11	0.05	−0.39 ± 0.15	0.24	−0.09 ± 0.19	−0.04	−0.29 ± 0.15
R^2		0.08		0.11		0.15		0.05
Sample size		92		100		85		112
Proportion surviving		0.84		0.73		0.64		0.67

'Pre-1976' includes all birds hatched in 1975 or earlier. For the 1982 drought period the 17 birds hatched in 1979 are included with the 1978 cohort. Significant values ($P < 0.05$) are underlined.

Table 4 Standardized selection differentials (s) and standardized selection gradients (β) over the drought of 1977, divided into three consecutive periods

	June–December 1976		December 1976 to June 1977		June–December 1977	
	s	$\beta \pm$ s.e.	s	$\beta \pm$ s.e.	s	$\beta \pm$ s.e.
Weight (g)	0.05	0.03 ± 0.04	0.28	0.23 ± 0.08	0.28	0.29 ± 0.12
Beak length (mm)	0.06	−0.01 ± 0.05	0.21	−0.17 ± 0.10	0.22	−0.04 ± 0.14
Beak depth (mm)	0.08	0.19 ± 0.07	0.30	0.43 ± 0.13	0.23	0.19 ± 0.19
Beak width (mm)	0.04	−0.15 ± 0.06	0.24	−0.19 ± 0.12	0.21	−0.14 ± 0.17
R^2		0.02		0.09		0.09
Sample size		640		442		194
Proportion surviving		0.69		0.44		0.50

The periods were defined by the times at which a census of surviving finches was taken. The data were not re-standardized from the first selection period. Significant values ($P < 0.05$) are underlined.

opposing selection in favour of small beak width. Given the high heritabilities of these characters[4,10], there is a strong potential for microevolutionary change between generations. The potential is realized[4], but only to a small degree for two reasons. First, there is a high positive genetic correlation, estimated to be between 0.9 and 1.0 (refs 4, 6), between the two beak traits that are selected in opposite directions. Second, net selection on beak depth and body weight over a whole life cycle is probably small because of opposing selection pressures at other stages of the life cycle, most notably in association with juvenile mortality: small individuals have a selective advantage over large individuals in their first 2–10 months, apparently for metabolic reasons[3].

Results of the study support an adaptive interpretation given

to beak size and shape in this population of Darwin's finches[6,11–13].

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Functions of the canal system in the rotaliid foraminifer, *Heterostegina depressa*

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Advanced fossil and living rotaliacean foraminifera are characterized by canal systems located within the walls of their plurilocular calcareous tests. Although these canals were described in detail during the nineteenth century[1–4], their biological functions have remained obscure. A study of the Recent nummulitid, *Heterostegina depressa*, has now illuminated the role of the canal system and allowed us to interpret similar structures seen in related foraminifera of Tertiary age. It proves to be a structure of fundamental importance for locomotion, growth, excretion, reproduction and protection.

Although foraminiferal tests are characterized by a multiplicity of shapes, wall structures and construction materials, in only a few taxa do we understand their functional roles well enough to demonstrate relationships to feeding methods or environments (substrates)[5,6]. The only group in which structural features can be explained as convergent adaptations to special modes of life are the larger symbiont-bearing benthic foraminifera (that is, members of the Miliolacea and Rotaliina). The transparency of the test wall and the high ratio of surface area to volume in those with flattened tests, provide the morphological basis for a highly advantageous algal–foraminiferal symbiosis[7–9]. We have examined the canal system within the symbiont-bearing Recent nummulitid, *H. depressa*, in which the test walls replace the primary aperture seen in other foraminifera.

H. depressa d'Orbigny inhabits the warm, shallow, nutrient-poor seas of the tropics and subtropics, where the light flux is relatively uniform throughout the year. This species exhibits marked physiological and morphological trimorphism: gamonts are up to 4 mm in diameter, while agamonts are up to 20 mm and schizonts up to 3 mm across. Although laboratory cultures[10] in which all three forms reproduced were a prerequisite for this investigation, specimens from the sea around Hawaii were also used.

In most multilocular foraminifera, successive chambers are connected by a single foramen, the last-formed chamber opening into the surrounding medium through the terminal foramen (aperture). Many species of rotaliid foraminifera have canal systems of different kinds within the chamber walls, and in genera such as *Operculina* these canals allows communication between the undivided chamber cavities and the lateral surfaces of the test. The subdivided chamber cavities (chamberlets) of *Heterostegina* communicate with the lateral surfaces in a similar manner. Nummulitids also have a three-dimensional network of canals (continuous with the rest of the canal system) within the thickened peripheral keel (marginal cord), and this opens into the ambient seawater.

It is known that nummulitids do not usually have true primary or secondary apertures[11], and few, if any, type descriptions of *Heterostegina* species establish their presence. This is not surprising, since the canaliculate marginal cord together with the canals of the chamber and chamberlet walls serve as an effective substitute. We have, however, observed apertures of various shapes and sizes in at least some individuals (see ref. 12).

The morphology of the canal system has recently been investigated by optical and scanning electron microscopy, and its genesis explained; it has also been used taxonomically[9,11,13,14]. The canal system permits the extrusion of pseudopodia from any point of the marginal cord, even when protoplasm has been retracted from the peripheral chambers. We regard this as its primary function, since it provides the animal with a physiological radial symmetry. In foraminifera lacking a canaliculate marginal cord, pseudopodia emerge only from a single point in the test (the aperture), and then only if the protoplasm has not been retracted from the last chamber. As the extrusion of pseudopodia is the primary function of the aperture in other groups of foraminifera, it seems likely that its secondary functions are also taken over by the canal system in the Nummulitidae. That this is so is demonstrated here for the first time.

In *H. depressa*, the terminal openings of the canals in the marginal cord function as a multitude of small primary apertures and extrude protoplasm which forms the template for the new chamber (Fig. 1). Although this process has been described previously[12], only since it was recorded on film[15] has the significance of the canal system been appreciated. Nummulitids such as *H. depressa*, *Heterocyclina tuberculata* Hottinger and *Operculina ammonoides* (Schröter) would probably be unable to construct large chambers with only a single exit for protoplasm.

H. depressa is nourished by photosynthates obtained from symbiotic algae contained within the cell, and by the continuous digestion of a proportion of the total population of symbionts[16]. Digestion occurs throughout the test, and waste products are transported by the protoplasm from older to younger chambers. Eventually, they are excreted through the openings in the marginal cord on the terminal chamber (Fig. 2) and through the aperture where this exists. The canal system thus provides for the removal of waste matter.

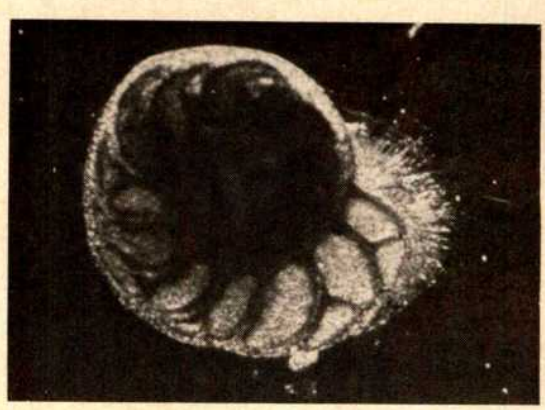


Fig. 1 Process of chamber formation in *H. depressa*. Protoplasmic filaments in large numbers emerge from the canals of the marginal cord of the youngest chamber in order to construct the template of a new chamber. Size of specimen 900 μm.

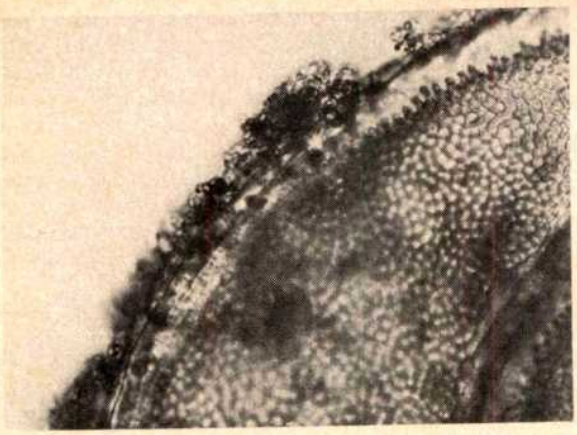


Fig. 2 Part of the lateral surface of the youngest chamber of *H. depressa* with accumulations of excrement on the marginal cord of the test. The 1–2 μm-sized grains have been excreted through the canals of the marginal cord.

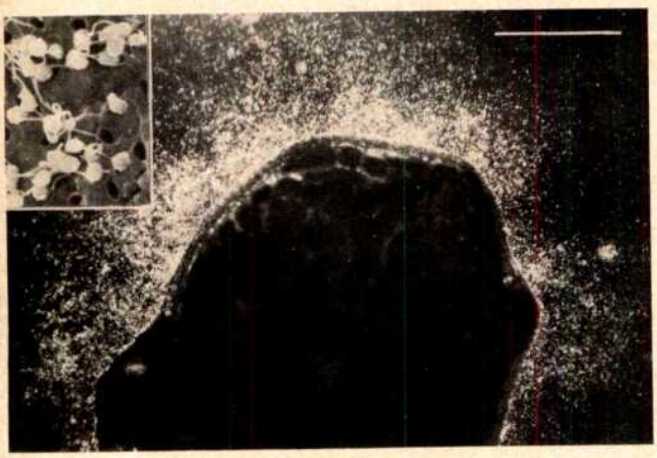


Fig. 3 Gamont of *H. depressa* from 100 m water depth off Honolulu, Hawaii, releasing gametes (scale bar, 420 μm). Inset: The scanning electron micrograph of the test surface shows pores and biflagellate gametes which were released through the canals. Size of the gametes 1 μm owing to shrinkage during fixation; size of living gametes 2.5 μm.

The canal system also functions during sexual reproduction. The 2.5-μm gametes are released mainly through the canal system of the marginal cord (Fig. 3). During asexual multiple fission of the schizont and agamont, the protoplasm, along with the symbionts, evacuates the test and subsequently divides into hundreds or thousands of daughter cells in the ambient seawater. Microcinematographic time-lapse photographs[17] have shown that the protoplasm emerges from the lateral surfaces of the parent test via the canal system (Fig. 4*a–c*). The symbiotic diatoms undergo severe deformation as they are transported through the 2–3-μm wide sutural canals. This is possible because they do not possess silica frustules but only membranous cell envelopes; normally, they are 8–11 μm long, 4–5 μm wide and very variable in shape[18]; they are thus well-adapted to this mode of transport. The outflow of the entire protoplasm through the long and narrow canal system takes a comparatively short time (38 min–4 h 16 min).

Throughout most of its life, *H. depressa* is covered by a transparent sheath attached to the substrate by elastic processes. This prevents transport in turbulent water and also protects the chambers during the growth phases which occur internally. Immature individuals leave their sheaths at intervals and replace them with larger ones[12,19,20]. Light microscope observations show that the secretion of the sheath lasts only a few hours, the material being passed through openings of the canal system onto the surface of the test. The canal system as a whole may therefore be said to provide the morphological basis for the rapid formation of this organic protective structure.

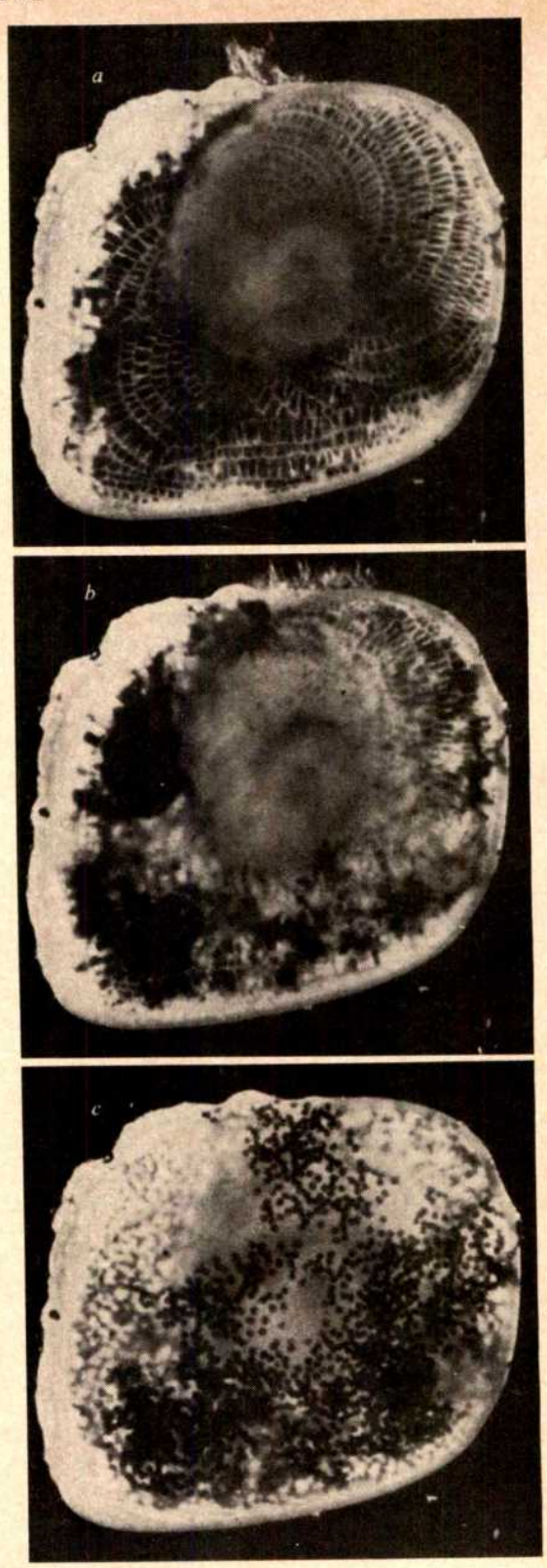


Fig. 4 Microcinematographic shots[17] of an agamont of *H. depressa* from 45 m water depth, Island of Maui, Hawaii (size 9.8 mm). The protoplasm emerges from the test and then divides into the daughter cells. *a*, The protoplasm has retracted from the peripheral part of the calcareous test, thus indicating the beginning of multiple fission. Its dark coloration, caused by the symbiotic diatoms, contrasts with the colourless walls of the chambers and chamberlets. *b*, The protoplasm has evacuated most of the test through the canal system, and now covers both lateral surfaces. *c*, The protoplasm has divided into the daughter cells. Juveniles lying below the maternal test are also visible. Duration of evacuation process 2 h 17 min.

The canal system thus proves to be important for all the principal biological functions of the protoplasm.

Although the flattened and translucent nummulitid test is well adapted to its function as part of a symbiotic association, it also encloses a large volume of protoplasm (plus symbionts) that has to communicate with the outside world. This connection is effected by the canal system which provides physiological continuity between the internal protoplasm and the surrounding seawater.

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Peripheral injury enhances central regeneration of primary sensory neurones

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The success of peripheral and fetal neural tissue in promoting outgrowth of axons from the adult mammalian central nervous system[1–4] has tended to focus attention on local interactions between extending axons and their environment. The contribution to axon regeneration of biochemical and morphological changes in injured neurones[5–7] is more difficult to evaluate. We report here that long spinal axons of primary sensory neurones are 100 times more likely to regenerate into peripheral nerve grafts if their peripheral axons are also cut. Regenerative behaviour at the axon tip seems to be strongly influenced by inducible events in the nerve cell.

In each of 11 rats, 6–8 weeks old, the dorsal columns were incised bilaterally after high cervical laminectomy and a segment from the right sciatic nerve was transplanted in the midline to the injured spinal cord. Such autogenous grafts provide a favourable milieu for spinal axons that usually fail to regenerate in their natural neuroglial environment[2,8,9]. Two to three months after the operation, retrograde tracing with horseradish peroxidase (HRP) was used to identify neurones with axons regenerating from the spinal cord (Fig. 1). Particular attention was paid to the L4 and L5 dorsal root ganglia (DRG), which contain many large neurones projecting centrally to the brain stem and peripherally to the sciatic nerve. Control experiments with crushing of grafts near the spinal cord at the time of injection of HRP excluded the possibility of false-positive labelling by diffusion or vascular spread. It was assumed that a neurone was labelled only if its spinal axon had entered a peripheral nerve graft and grown several millimetres or more within it. On the left, the L4 and L5 DRG usually contained no labelled neurones, and the most per pair was 10. On the right, all L4 or L5 DRG contained labelled neurones and the maximum for a pair of ganglia was 682. In all 22 matches between L4 or L5 ganglia the count was always higher on the right than on the left. The mean count per ganglion on the right (114) was more than 100-fold greater than on the left (<1). To provide a rough estimate of the size of the pool of lumbar sensory neurones with axons projecting to the vicinity of the dorsal column nuclei, a mixture of wheat germ agglutinin and HRP was injected into the dorsal columns at high cervical level in normal rats. The highest number of labelled neurones counted in a single L5 DRG was 840. In DRG of some grafted animals, the number of neurones with regenerating spinal axons exceeded 50% of this count.

Similar grafting and labelling techniques were used on four other groups of rats (Fig. 2*a*) receiving different combinations of treatments to the two sciatic nerves. When neither sciatic nerve was cut (in Lewis rats with isogenous grafts), almost no regeneration was detected from lumbar DRG; when both sciatic nerves were cut, the mean count for labelled neurones in L4 + L5 DRG on each side was 385 and the difference between counts on the two sides was not significant. The latter result excludes a systematic bias in positioning of the grafts or a strong tendency for axons in one dorsal column to regenerate into an ipsilateral rather than contralateral nerve segment. Sham operation or crushing of the sciatic nerve was much less effective than nerve transection in evoking spinal regeneration; after either procedure, counts of labelled neurones in ganglia on the appropriate side averaged less than 10% of those on the side from which the graft was taken. In 70 C8 DRG, the mean and range of the number of labelled neurones were 1 and 0–7, respectively. Sciatic nerve injury did not seem to influence growth into grafts of axons arising from contralateral lumbar DRG or cervical DRG.

Finally, injuries to peripheral and spinal axons were staggered in time by grafting a segment of one sciatic nerve to the spinal cord and cutting the other sciatic nerve 1 or 4 weeks earlier or later (Fig. 2*b*). Sciatic nerve injury was comparably effective in inducing central regeneration when it preceded rather than coincided with grafting. However, after a delay of as little as 1 week, cutting the second sciatic nerve resulted in significantly less regeneration from the corresponding dorsal column. It appeared that a propensity for regeneration persisted in sensory neurones for at least 1 month after sciatic nerve transection but that the environment near the junction between spinal cord and nerve segment became less conducive to axon outgrowth within 1 week of grafting.

After injury at high cervical level, spinal axons of neurones with cell bodies in L4 and L5 ganglia regenerated in abundance only if the ipsilateral sciatic nerve was also cut. The virtual absence of regeneration in basal conditions indicates that normal cellular function in these nerve cells does not usually support regeneration of their spinal axons injured at a distance from their somata. The enhancement of regeneration by a stimulus remote from the site of axon growth suggests that critical cellular responses affecting the entire neurone have been induced. Under other circumstances, events in these perikarya are of questionable significance during axon regeneration; for example, axons in crushed dorsal roots regrow towards the spinal cord with no obvious chromatolysis or change in axoplasmic transport[10–12]. The neuronal reactions[6,13,14] that promote regeneration of spinal axons, and the retrograde signals[15–17] that trigger these cellular responses are unknown.

Priming of peripheral nerve regeneration by a preceding crush injury[18] differs from the enhancement seen here in that conditioning and testing lesions are separated temporally rather than spatially. A 'pruning' effect that has been described after a single lesion to branching axons of invertebrates and vertebrates[19–21] involves proliferation of uninjured fibres as

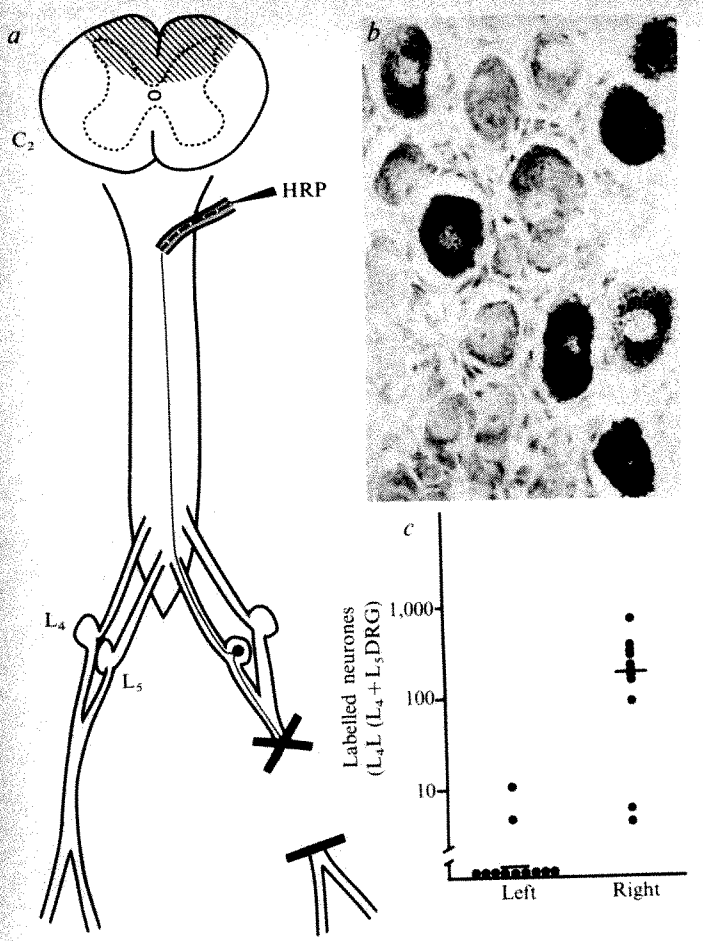


Fig. 1 *a*, Diagram showing experimental procedure. Experiments were performed on female rats weighing ~150 g and anaesthetized with pentobarbital. Under an operating microscope, the second cervical lamina was removed and a symmetrical transverse incision approximately 1 mm deep was made in the dorsal columns. One end of a 2-cm segment of the right sciatic nerve was apposed to the injured spinal cord in the midline with a stitch through the meninges. The left sciatic nerve was uninjured. Two to three months after grafting, the other end of each graft was exposed subcutaneously in the suboccipital region and injected with a total of 1 μl 20% HRP through a glass micropipette. Two days later the rats were perfused with buffered 3% glutaraldehyde and serial sections, 20 μm thick, were cut through the L4 and L5 DRG, reacted with tetramethylbenzidine[29] and counterstained with neutral red. *b*, Representative HRP-containing neurones in an L4 DRG on the right side. *c*, Labelled neurones were counted in the L4 and L5 DRG of 11 rats. To avoid double counting, only neurones with visible nucleoli were counted. The error due to split nucleoli was ignored. The difference between numbers of labelled neurones in DRG on the left and right was highly significant ($P < 0.001$) to either Mann–Whitney or Student's *t* statistical testing. Bars represent mean sums of labelled neurones in L4 and L5 DRG.

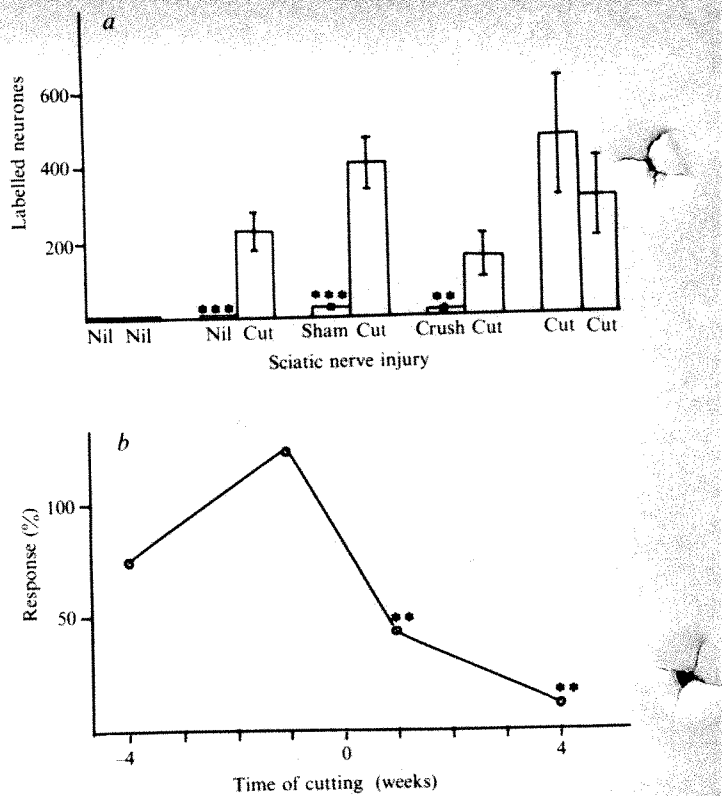


Fig. 2 *a*, Sums of labelled neurones in the L4 and L5 DRG recorded for left and right sides in five groups of rats. Nil, no operation on leg; sham, sciatic nerve exposed but not cut; cut, sciatic nerve cut at the origin of the nerve to the biceps; crush, sciatic nerve crushed for 15 s with jeweller's forceps at the level of the nerve to the biceps. Means ± s.e.m., $n = 6$–11. Asterisks indicate significantly fewer neurones than on the cut side: ** $P < 0.01$, *** $P < 0.001$ (Mann–Whitney *U*-test). *b*, In four groups of rats ($n = 6$–9), the left sciatic nerve was cut 1 or 4 weeks before or after grafting of a segment of the right sciatic nerve to the dorsal spinal cord at high cervical level. For each point, the sum of labelled neurones on the left is expressed as a percentage of the sum on the right for the entire group. ** Significant difference between the two sides ($P < 0.01$, Mann–Whitney *U*-test).

opposed to regeneration of cut axons. Possibly, all three phenomena reflect similar molecular events. Other transganglionic effects of peripheral axotomy on central axons of mammalian sensory neurones include changes in fibre diameter, conduction velocity, fast axoplasmic transport and content of peptides and enzymes[14,22–26]. Reasons for the difference between the effects of peripheral nerve crush and transection on the function of sensory neurones[5,23,27,28] are unclear.

Without peripheral axotomy, spinal axons of primary sensory neurones regenerate into peripheral nerve grafts no better than, for example, rubrospinal or spinocerebellar axons[9]. Very few axons from dorsal or lateral spinal tracts grow into peripheral nerve segments after injury more than a few centimetres from their cell bodies. When sensory neurones are stimulated by peripheral nerve transection, their spinal axons regenerate much more vigorously than without a second injury. The hypothesis that neurones lying entirely within the central nervous system might be extrinsically induced in a similar manner is exciting to contemplate but difficult to test.

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Vitamin E protects against retinopathy of prematurity through action on spindle cells

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In the premature infant, exposure of the incompletely vascularized retina to increased oxygen tension can result in the development of a blinding disease, retinopathy of prematurity (ROP). Despite the judicious curtailment of oxygen, the incidence of ROP is on the increase due to the technological advances that have improved the survival of the very young preterm infant[1]. Six clinical trials[2–7] have documented the efficacy of vitamin E supplementation in suppressing the development of severe ROP[8–10], but the mechanism of this protection has remained unknown. This report proposes that spindle cells, mesenchymal precursors of the inner retinal capillaries, are the primary inducers of the neovascularization associated with ROP. Exposure of spindle cells to elevated oxygen tension increases their gap junction area. This early morphologic event immediately halts the normal vasoformative process and eventually triggers the neovascularization that is observed clinically 8–12 weeks later. Vitamin E supplementation above the deficient plasma levels of these infants[11] suppresses gap junction formation and clinically reduces the severity without altering the total incidence of ROP.

The database is 69 pairs of whole-eye donations from infants who were live-born, anomaly-free at 20 to 31 weeks gestational age and ≤1500 g birth weight, and who survived from 30 min to 20 weeks. Twenty-two infants received minimal or no vitamin E supplementation (control infants) with mean plasma vitamin E levels of 0.3–0.6 mg%; 47 infants received vitamin E supplementation (treatment infants) with mean plasma vitamin E levels of 1.2–3.3 mg%, which are within the adult physiologic range[12,13]. All whole eye donations contained avascular regions in all quadrants of the retina and were processed for light[14], transmission[15], and scanning electron microscopy. Light micrographs of continuous areas from the optic disk to the ora serrata of both the nasal and temporal hemispheres were assembled into montages. Along these montages, the following parameters were determined morphometrically: percentage of vessels with necrotic endothelial cells, percentage of spindle cells in a migrating or canalizing configuration, and percentage volume of the nerve fibre layer occupied by spindle cells[14]. Gap junction surface area between adjacent spindle cells was calculated from 40,000X electron micrographs using a Zeiss Videoplan and expressed as the percentage of the spindle plasma membrane surface area that was differentiated into gap junctions. All P values were calculated from a paired Student's t-test. All data are given for only the temporal hemisphere.

The morphometric analyses allow the role of the spindle cells in retinal vasoformation to be elucidated. As retinal development (Fig. 1*a*) and stratification (Fig. 1*b*) proceed peripherally from the optic disk, an apron of interdigitating spindle cells emerges from the adventitia of the hyaloid artery and migrates behind an advancing point of retinal development (Fig. 1*c*). The fusiform spindle cells within the migrating apron (Fig. 2*a*) are randomly oriented within the plane of the nerve fibre layer (Fig. 2*b*). Central to the fringe of the migrating arpon, nascent retinal blood vessels are formed by canalization of spindle cells (Fig. 2*c*). The canalization process involves formation of a lumen within a solid cord of spindle cells. Eventually erythrocytes from the formed capillaries penetrate the lumen (Fig. 2*d*). This entire sequence of migration, canalization, and endothelial differentiation normally occurs in the hypoxic (P_{O_2} = 25 mmHg)[16] *in utero* environment.

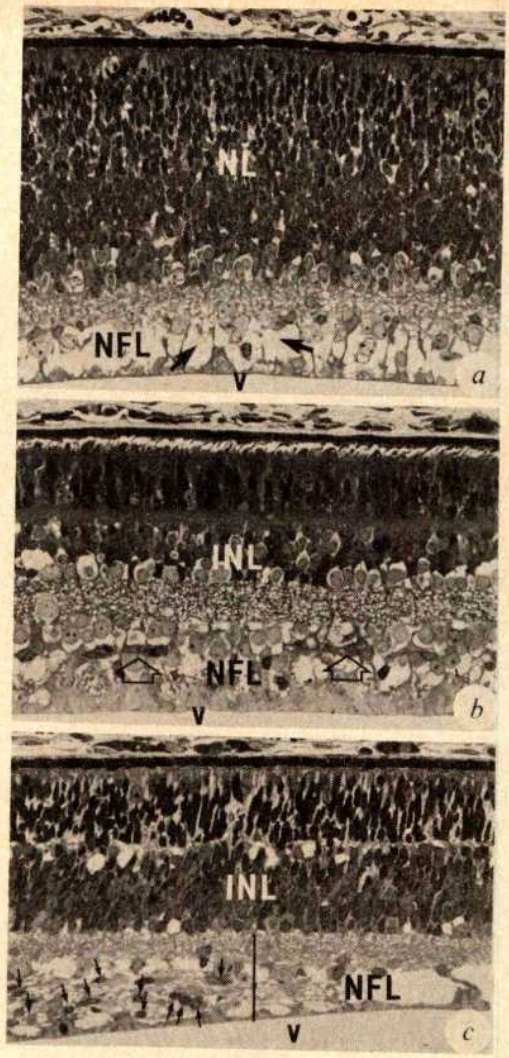


Fig. 1 Light micrographs (×240) demonstrating the relationship between the state of retinal development and the invasion of spindle cells into the nerve fibre layer. *a*, Undifferentiated midperipheral retina with primitive outer neuroblastic layer (NL) and no spindle cells within the nerve fibre layer (NFL) which is laced with cystoid spaces (→). *b*, Differentiated central retina in which the nerve fibre layer (NFL) contains developing capillaries (⌓). *c*, Apron of spindle cells (⇉) within the nerve fibre layer (NFL). The migration ceases at a distinct point (—) of retinal development. Inner nuclear layer (INL), vitreous (V).

The gap junction area of the spindle cells (Fig. 3) is modulated by oxygen tension, vitamin E supplementation, and gestational age. For the first four days post partum (14 control and 13 treatment infants), the the average gap junction area remains low (4.6 ± 2.1, range <1–12.1) (Figs 3*a*, 4), with spindle cells occupying <16% of the nerve fibre layer volume. These spindle cells are in migrating and canalizing configurations. In the absence of vitamin E supplementation (8 control infants), there is a significant (P = 0.0005) increase in gap junction area (Fig. 3*b*) as early as four days post partum (Fig. 4*a*). There is a cessation of canalization, and severe ROP develops in this group around 9 weeks post partum[5–7]. Despite the increase in gap junction area, the spindle cells still occupy <13% of the nerve fibre layer volume until after the development of severe ROP. The down modulation of gap junction area (>70 days post partum) (Fig. 4*a*) is a late event that correlates with stacking

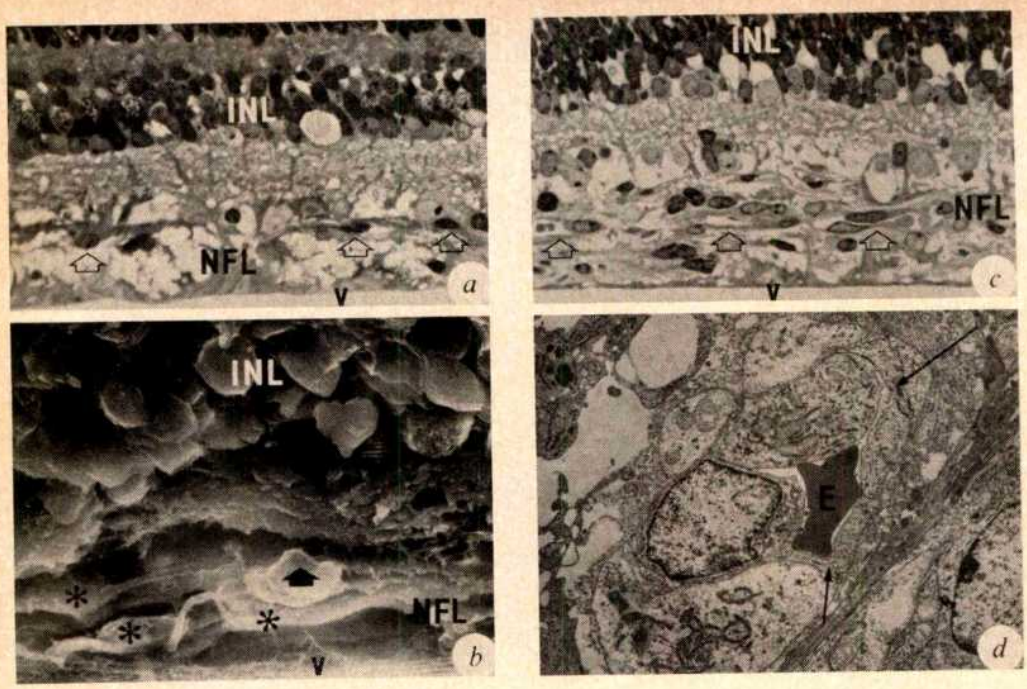


Fig. 2 Migrating and canalizing spindle cells within the developing human retina. *a*, Light micrograph (×435) of migrating spindle cells (⌓) forming an anastomising apron within the nerve fibre layer (NFL). *b*, Scanning electron micrograph (×2,250) of the migrating, fusiform spindle cells which are randomly oriented within the plane of the NFL. Contrast those spindle cells lying in a plane parallel to the scanned surface (✳) with those at right angles (◆). Inner nuclear layer (INL), vitreous (V). *c*, Light micrograph (×750) of canalizing spindle cells (⌓) posterior to the advancing apron in the NFL. *d*, Electron micrograph (×3,750) of canalizing spindle cells with erythrocyte (E) penetration into the lumen and marginal flap formation (←).

of the spindle cells such that they occupy 30–54% of the nerve fibre layer volume. Vitamin E supplementation suppresses gap junction formation, but this suppression is strongly dependent upon gestational age. In treatment infants ≤27 weeks (18 infants) (Fig. 4*b*), there is a lag period of approximately 16 days post partum in which minimal increases in gap junctions occur. Following this lag, there is a rapid increase in gap junction area to a level equivalent ($P = 0.3$) to that of unsupplemented infants. There is a parallel cessation of spindle cell canalization, but development of severe ROP in this group is delayed to around 12 weeks post partum[5–7]. In all treatment infants shown in Fig. 4*b*, the spindle cells occupy <14% of the nerve fibre layer volume. In treatment infants ≥28 weeks gestational age (Fig. 4*c*) (16 infants), gap junction formation is suppressed, canalization of spindle cells continues, spindle cells occupy a minimal volume of the nerve fibre layer (<13%), and no severe ROP develops. Thus, while continuous supplementation in infants ≥28 weeks gestational age suppresses gap junction formation and the development of severe ROP, supplementation in infants ≤27 weeks gestational age delays the onset of gap junction formation but does not diminish the severity of ROP in the highest risk infants. Vitamin E supplementation delays the down modulation of gap junction area and the clinical development of severe ROP (Fig. 4*a*, *b*).

Based on these ultrastructural observations, the following mechanism of the induction and suppression of ROP is proposed. When the retinas of high risk premature infants are exposed to relative hyperoxia, the elevated oxygen tension induces the formation of gap junctions between adjacent spindle cells. The gap junctions are the first morphologic evidence of oxygen toxicity, and their formation results in the subclinical cessation of spindle cell migration and canalization. The ionic coupling of spindle cells via gap junction formation changes the cells from potential endothelial precursors to sites of synthesis and release of angiogenic factors. The angiogenic factors trigger neovascularization from existing vessels over the next 8–12 weeks. Vitamin E supplementation to adult physiologic levels (1.1–3.3 mg%) suppresses gap junction formation by protecting the spindle cells from oxygen related damage, and thus reduces the severity of ROP.

This proposed mechanism of ROP induction can explain four clinical realities regarding the development, suppression, and treatment of this disease: (1) The clinical observation that

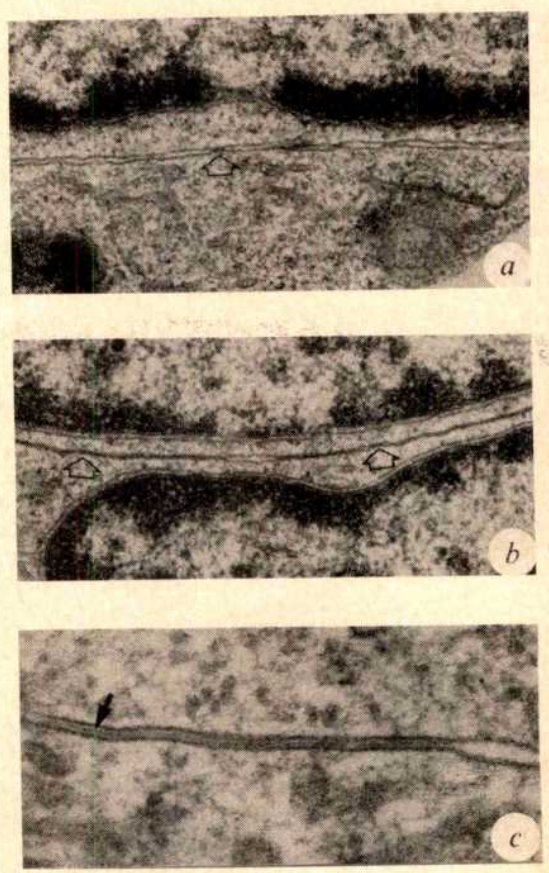


Fig. 3 Gap junctions between adjacent spindle cells. *a*, *b* Are electron micrographs (×32,000) demonstrating the plasma membrane surface area changes. *a*, Punctate close plasma membrane appositions (⌓); such spindle cells are actively migrating and canalizing. *b*, Extensive close plasma membrane appositions (⌓); such spindle cells have ceased migrating and canalizing and are primed to induce neovascularization from nascent capillaries. *c*, At higher magnification (×110,000), the close plasma membrane appositions are resolved as gap junctions with substructure within the 20–30 Å gap (→).

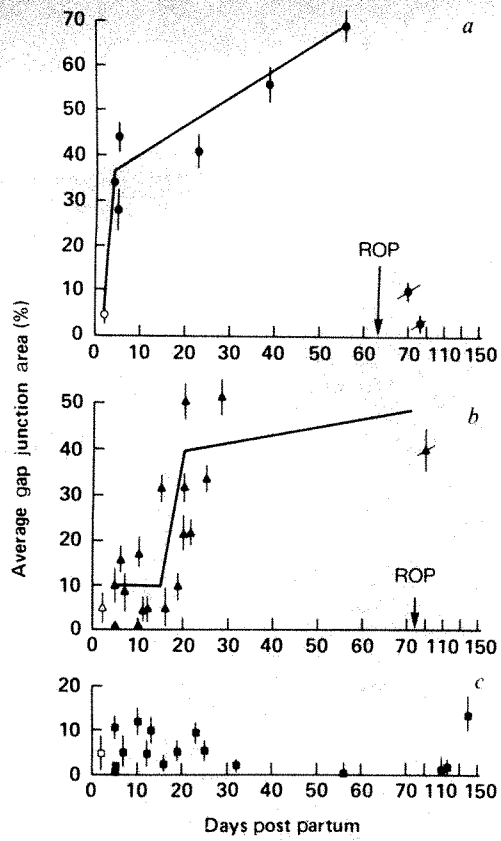


Fig. 4 Average gap junction area (%) ± s.e.m. as a function of days post partum. *a*, Control infants, $n = 8$ (●). *b*, Treatment infants ≤27 weeks gestational age, $n = 18$ (▲). *c*, Treatment infants ≥28 weeks gestational age, $n = 16$ (■). For each group, the average gap junction area in equivalent infants who survived for <4 days post partum (○, 14 infants; △, 9 infants; and □, 4 infants) is plotted. The three infants with clinically documented severe ROP are represented by ●, and ▲. The time when severe ROP develops for each group (arrows in *a*, *b*) is based on the results of clinical studies which enrolled 418 high risk preterm infants[5–7].

vitamin E supplementation is not efficacious unless initiated at birth[4–10] is explained by the ultrastructural observation that gap junctions can increase as early as four days post partum; thus, antioxidant protection is needed concomitantly with the increase in oxygen tension. (2) Although vitamin E supplementation suppresses gap junction increases in infants ≥28 weeks gestational age, it is not an absolute suppression; thus, the severe blinding grades of ROP are suppressed[5–7] while the total incidence of all grades of ROP remains constant. (3) Severe ROP may develop in infants ≤27 weeks gestational age despite continuous supplementation because vitamin E only transiently suppresses increases in gap junctions in these infants. After a lag period of approximately 2 weeks, gap junctions may increase to a level commensurate with that of unsupplemented controls. This lag correlates with the delayed onset of severe ROP in the highest risk infants[5–10]. (4) Ablation of only the avascular peripheral retina has been utilized empirically to induce regression of severe ROP before retinal detachment[17–19]. The proposed mechanism of ROP induction would predict the success of this procedure, since it involves the destruction of the gap-junction-linked spindle cells. In contrast, ablation of the proliferating vessels or the vascular:avascular interface leads to vitreous haemorrhage and retinal separation[20–22]. The ultrastructural explanation of the failure of this procedure is that the gap-junction-linked spindle cells remain operant and continue to secrete angiogenic factors and induce neovascularization.

The spindle cell mechanism for the induction of ROP contrasts with that proposed by Ashton[23] and Patz[24,25] in which vasoconstriction, vaso-obliteration, endothelial necrosis, and hypoxia were believed to be the initiating events. No endothelial cell necrosis was observed in any of the 69 pairs of whole eye donations in this study. Only the spindle cell concept explains the natural history of ROP and the gestational age dependence of the efficacy of vitamin E supplementation in suppressing the development of severe ROP. Furthermore, it rationalizes surgical intervention that destroys the peripheral inducers of this developmental retinopathy.

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Correlation between inositol phospholipid hydrolysis and substance P receptors in rat CNS

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The undecapeptide substance P is a neurotransmitter candidate in the mammalian central and peripheral nervous system[1]. Although the distribution of substance P-like immunoreactivity within the central nervous system (CNS) is well established[2], the recent identification and autoradiographic localization of specific substance P-binding sites has revealed numerous areas of mismatch between peptide levels and numbers of such sites[3–6]. Previous studies have shown that substance P stimulates the hydrolysis of inositol phospholipids in peripheral tissues and in the hypothalamus[7,8], probably through stimulation of a polyphosphoinositide-specific phospholipase C (refs 9–11). Inositol phospholipid hydrolysis has been implicated in the mobilization of cytosolic calcium following receptor activation in several neurotransmitter and hormonal systems[12]. We have therefore investigated the distribution of ^{3}H-labelled substance P binding sites within various rat brain regions and correlated this with the rate of substance P-induced hydrolysis of inositol phospholipids in the same areas of the CNS. We found that the rate of inositol phospholipid hydrolysis was proportional to the number of binding sites specific for ^{3}H-substance P, suggesting that binding sites revealed by ^{3}H-substance P autoradiography correspond to functional substance P receptors.

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The distribution of specific ^{3}H-substance P-binding sites throughout the rat brain was initially determined. Adult male Sprague–Dawley rats were killed, their brains removed, frozen on dry-ice and 20-μm sections cut using a cryostat in either the horizontal or the coronal plane. They were apposed onto gelatinized glass slides and stored in boxes with desiccant for 48 h at −20 °C. The sections were then preincubated at room temperature for 10 min in a solution containing 0.005% polyethyleneimine in 50 mM Tris-HCl, *p*H 7.40, followed by incubation in the presence of ^{3}H-substance P. Nonspecific binding was

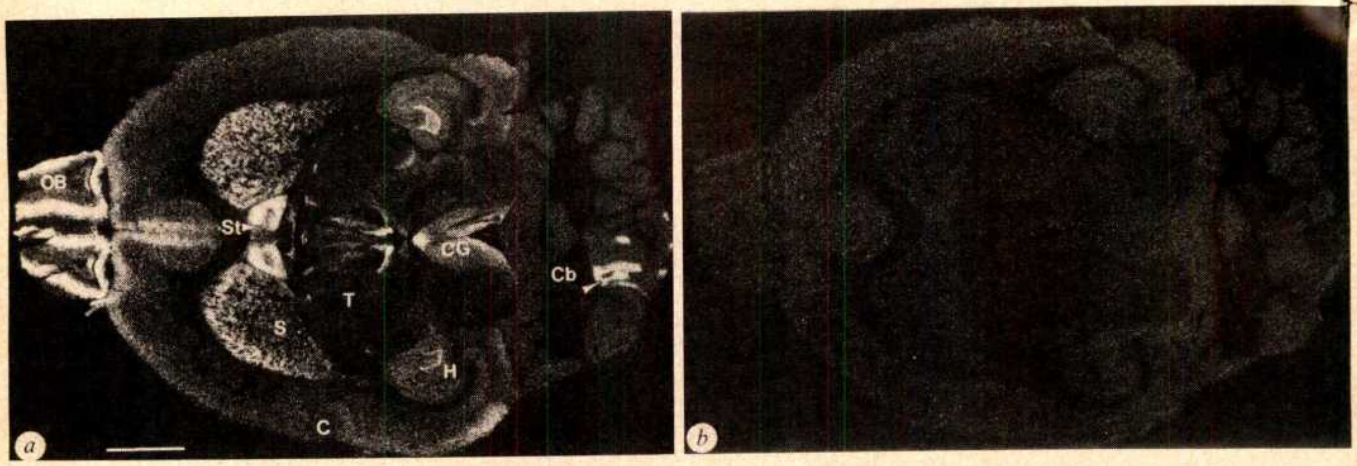


Fig. 1 ^{3}H-substance P-receptor distribution in the rat brain. *a*, A typical darkfield photomicrograph of ^{3}H-substance P binding in a horizontal brain section. The light areas are silver grain concentrations indicative of specific binding. *b*, A darkfield photomicrograph of an adjacent control section treated identically to *a*, except that 1 μM substance P (Peninsula) was added to the incubation solution. Note the heavy labelling in several areas, such as the external plexiform layer of the olfactory bulb (OB), the striatum (S), the septum (St), the central grey (CG) of the midbrain and folia IX and X of the cerebellum (Cb, arrowhead), moderate labelling in the cortex (C) and hippocampus (H) and absence of label in the thalamus (T). Scale bar, 2.5 mm.

Methods: Horizontal 20-μm sections were preincubated at room temperature for 10 min in a solution of 50 mM Tris-HCl (*p*H 7.4) containing 0.005% polyethyleneimine (Sigma). The slides were then incubated for 10 min in a solution of 50 mM Tris-HCl (*p*H 7.4) containing 0.02% bovine serum albumin (Sigma, radioimmunoassay grade), chymostatin (2 mg l^{-1}; Sigma), leupeptin (4 mg l^{-1}; Sigma), bacitracin (40 mg l^{-1}; Sigma) and increasing concentrations of [2-L-prolyl-3,4-^{3}H(*N*)] substance P (specific activity 26 Ci $mmol^{-1}$, NEN). Nonspecific binding was defined as binding in the presence of 1 μM substance P (Peninsula) and specific binding obtained by subtracting the nonspecific binding from the total binding. In these incubation conditions >95% of ^{3}H-substance P eluted at the position of intact substance P on reverse-phase HPLC and the specific binding reached a plateau after about 8 min. The slides were rinsed with four washes of ice-cold 50 mM Tris-HCl (*p*H 7.4), 5 s each, and four washes of water, 5 s each, dried in a stream of cold air and stored overnight in desiccant. The slides were then placed in apposition to a tritium-sensitive film (^{3}H-Ultrofilm, LKB). After 12 weeks the film was developed using Kodak D-19 developer and photographed under darkfield illumination. To estimate the relative density of specific ^{3}H-substance P-binding sites we used microdensitometry combined with the tritium-sensitive film, as described previously[14]. The exposed film was placed on the stage of a Leitz orthoplan microscope equipped with a Vario-Orthomat camera system. Direct reading of the voltage from the photosensitive cell served as a density reading. Calibration experiments were used to determine in which region the density was linearly dependent on the isotope concentration and the measurements were performed in that region. The densitometer was able to resolve density differences of the orderof 0.02 *A* units and readings were taken from an area of 200 μm diameter. Background labelling was defined as the ^{3}H-substance P binding in the presence of 1 μM substance P.

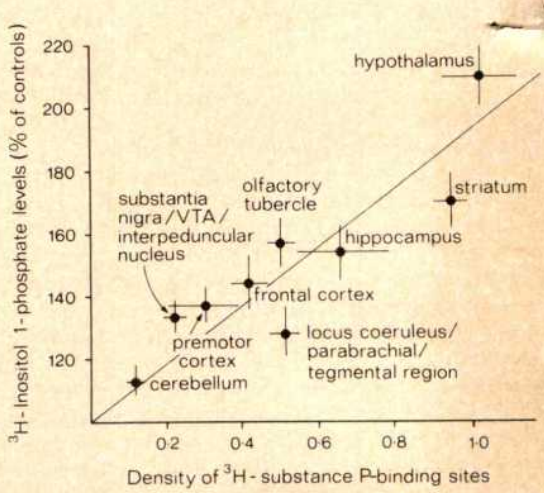


Fig. 2 The density of substance P receptors plotted against the magnitude of substance P-induced inositol phospholipid hydrolysis in various regions of the rat brain. The density of substance P receptors was determined using autoradiography coupled to a tritium-sensitive film and analysed using microdensitometry, as described for Fig. 1. All readings were taken from sections incubated in and exposed to the same conditions. To quantify the relative density of receptors, we designated the highest area measured (the hypothalamus) as 1.0 and ranked the others accordingly. The mean ± s.e.m. of at least four determinations throughout the area under examination was taken.

Methods: The substance P-induced hydrolysis of inositol phospholipids in various regions of the rat central nervous system was determined according to Berridge *et al.*[15]. Sprague–Dawley rats (150–200 g) were killed, the brain removed, blocked and placed into an oxygenated solution of artificial cerebrospinal fluid (CSF, composition in mM: NaCl, 124; KCl, 2; KH_2PO_4, 1.25; $MgSO_4$, 2; $CaCl_2$, 2; $NaHCO_3$, 25 and glucose, 11). Sections (300 μm) were cut using a vibratome (Oxford Instruments) and the appropriate brain area dissected out using microscissors; one side was used for stimulation with substance P and the contralateral side served as the control. Sections were placed in CSF solution for 2 h at 37 °C and then transferred to 500 μl of prewarmed oxygenated CSF to which 1 μCi myo-[2-^{3}H]inositol (specific activity 10–20 Ci $mmol^{-1}$) (Amersham) and 12 mM LiCl had been added. One hour later 1 μM substance P (Peninsula) was added and the tubes were incubated for another 30 min. The incubation was terminated by addition of 500 μl trichloroacetic acid and the tubes were left for 15 min on ice. Following a 15-min centrifugation at 2,000*g*, 700 μl of the supernatant was removed. The latter was then washed three times with 4 ml of ether, with the ether phase being discarded after each wash, and 700 μl of 20 mM Tris-HCl containing 5 mM EDTA was added to each tube. Tritiated inositol 1-phosphate was separated from other water-soluble metabolites by application of the sample to columns containing 1 ml of Dowex 1-X8 (Sigma) in the formate form. The columns were washed twice with 8 ml of 60 mM ammonium formate/5 mM disodium tetraborate and ^{3}H-inositol 1-phosphate was eluted with 8 ml of 200 mM ammonium formate/100 mM formic acid and the radioactivity determined by liquid scintillation spectrometry. The levels of ^{3}H-inositol 1-phosphate are expressed as per cent of controls (taken as 100). In a typical experiment on the hypothalamus the control value was 1,844 ± 127 d.p.m. ^{3}H-inositol 1-phosphate per tissue slice ($n = 6$). The results are expressed as the means ± s.e.m. of triplicate determinations from four separate experiments.

defined in the presence of 1 μM substance P. Preliminary experiments had shown that, in the absence of polyethyleneimine, >95% of the ^{3}H-substance P binding was to the glass around the tissue rather than to the tissue itself, while specific binding reproducibly amounted to 60% of the total binding in the presence of polyethyleneimine. Following incubation in the presence of ^{3}H-substance P, tissue sections were apposed to a tritium-sensitive film.

Previous experiments[13] had shown that ^{3}H-substance P bound to a single class of binding sites with an equilibrium dissociation constant of 1.1 nM and a maximum number of binding sites of 15 fmol per mg tissue. The fragment potencies agreed well with results from studies on the CNS which had used tissue homogenate binding assays for substance P[4,5].

The correlation between the autoradiographic distribution of specific ^{3}H-substance P-binding sites (Fig. 1) and a biochemical response linked to substance P-receptor occupancy was investigated by incubating vibratome sections from various brain regions in the presence of 1 μM substance P and measuring the rate of inositol phospholipid hydrolysis. When the magnitude of substance P-stimulated inositol phospholipid hydrolysis was plotted against the mean number of specific substance P-binding sites in various brain regions, an excellent correlation was obtained (correlation coefficient 0.96, as judged by regression analysis; Fig. 2).

The present results therefore indicate that, while the distribution of substance P receptors in the CNS does not correlate with the distribution of substance P-like immunoreactivity[3–6], the increase in inositol phospholipid hydrolysis effected by substance P is directly proportional to the number of specific ^{3}H-substance P-binding sites present in various brain regions. Given the interest in substance P agonists and antagonists, this measure of inositol phospholipid hydrolysis should provide a reliable biochemical assay for substance P receptors in the CNS.

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H–2-linked low-molecular weight polypeptide antigens assemble into an unusual macromolecular complex

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The major histocompatibility complex (MHC) is a cluster of tightly linked genes whose products are of central importance in the functioning of the immune system. Class I and II MHC antigens are integral membrane proteins which regulate cell-surface interactions between T cells and their targets[1–3], while class III antigens are components of the complement system of serum proteins[4]. All available evidence indicates that the structure and function of the MHC and its gene products are highly conserved among species (for review, see ref. 5). We recently reported[6] the existence in murine cells of a fourth class of MHC-linked polypeptides which are biochemically and genetically distinct from previously identified MHC gene products: BALB.B anti-BALB/c (anti-H–2^d) antiserum immunoprecipitates a set of 16 cytoplasmic low-molecular weight polypeptides (LMP) from BALB/c spleen cells and from the WEHI-3 cell line. The production of these peptides is coordinately regulated (by immune interferon) with the production of the class I and II MHC antigens[7,8], suggesting that they too are functionally relevant to the immune system. We demonstrate here that these 16 polypeptides are associated with one another *in vivo* as a very large (580,000-molecular weight, M_r) noncovalent complex. The unusual nature of this complex has allowed the non-immunochemical identification of similar complexes from (serologically negative) H–2^b murine cells and from a human cell line. Thus, LMP antigens display two properties in common with other MHC antigens: they are both polymorphic and genetically conserved across species.

To determine whether our antiserum recognizes 16 different chains, or whether some of the LMP are associated with one another *in vivo*, extracts of ^{35}S-methionine-labelled WEHI-3 cells were size-fractionated by gel filtration before immunoprecipitation. As Fig. 1 shows, all of the LMP activity elutes from the column as a single peak of ~580,000 M_r. No LMP activity is detectable in the lower molecular weight column fractions. This peak of LMP activity is clearly distinct from the void volume peak (Fig. 1) which, in the absence of detergent, contains all of the (aggregated) H–2 and Ia antigens present in the original sample (data not shown), and presumably any other nonspecifically aggregated material. More importantly, the LMP peak is bounded on both sides by molecular weight markers. The results of gel filtration using gels of different exclusion limits and with different buffers (Table 1) were always consistent with the data shown in Fig. 1. This experiment therefore strongly suggests that all 16 LMP polypeptides are specifically associated with one another *in vivo* in the form of a high-molecular weight complex.

Figure 2*b*, *c* compares the peak LMP fraction from the gel filtration column with an LMP immunoprecipitate, and shows that nearly all of the peptides found on the portion of the gel shown ($M_r \simeq 12{,}000$–$35{,}000$) in Fig. 2*c* are LMP chains. Note, however, that there is a large amount of labelled material in the higher molecular weight portion ($M_r > 50{,}000$; not shown) of the gel in Fig. 2*c* which does not appear in LMP immunoprecipitates and is therefore not part of the LMP complex. In fact, the purity of the peak chromatography fraction can be roughly estimated by comparing the total number of ^{35}S c.p.m. in the fraction with the number of c.p.m. in the LMP chains (determined by immunoprecipitation). By this criterion, the LMP comprise only approximately 1–2% of the total material in the fraction.

Thus, a single step of gel filtration yields a sample of LMP which is sufficiently pure to be visualized cleanly by two-dimensional polyacrylamide gel electrophoresis (PAGE) without prior immunoprecipitation. We have also used this technique to analyse the remaining column fractions in Fig. 1 (data not shown). No LMP chains are detectable in any of the fractions except that corresponding to the LMP peak shown, indicating that the individual LMP chains do not exist in an uncomplexed form. In addition, for all of the fractions in which LMP chains are detectable, the relative intensity of the individual chains seems to be constant. These experiments therefore suggest

Table 1 Gel filtration of LMP complexes in various conditions

Sephacryl S200	TBS + 0.5% NP40	>250,000*
Sephacryl S200	DPBS	>250,000*
Sephacryl S200	DPBS + 0.5 M NaCl	>250,000*
Sepharose CL-6B	DPBS	≃580,000
Sepharose CL-6B	DPBS + 0.5 M NaCl	≃580,000

TBS, Tris-buffered saline; DPBS, Dulbecco's phosphate-buffered saline (Gibco); NP40, Nonidet P-40.
* All LMP activity was present in the column void volume peak.

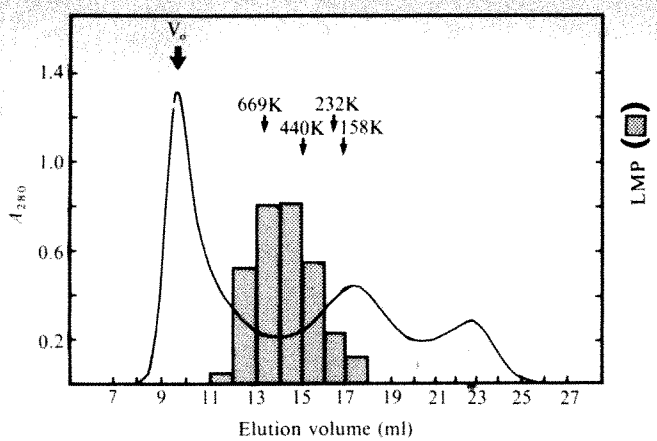


Fig. 1 Immunoprecipitation of LMP antigens from size-fractionated WEHI-3 extracts. Cells were grown and labelled with ^{35}S-methionine as described previously[6]. Before labelling, cells were grown for 2–4 days in the presence of concanavalin A-activated spleen cell supernatants to induce maximal LMP expression (see ref. 8). For gel filtration experiments, the washed labelled cells were resuspended in Dulbecco's phosphate-buffered saline (Gibco) containing 0.1% sodium azide (DPBS) and lysed by five cycles of freeze/thaw. Membranes and insoluble cellular debris were pelleted by centrifugation, and the clear supernatants were stored at −70 °C until use. A 0.5 × 30 cm Sepharose CL-6B column calibrated with molecular weight standards (Pharmacia) was used. Arrows designate the elution volume of molecular weight markers: V_0, void volume; 669,000 (669K), thyroglobulin; 440K, ferritin; 232K, catalase; 158K, aldolase. Samples (0.5 ml) were run in DPBS or DPBS containing 0.5 M NaCl, and 1-ml fractions were collected. Immunoprecipitations were performed on column fractions as described previously[6], except that 500 μl of sample and 50 μl of antiserum were used. For samples to be analysed without immunoprecipitation, 10 μl of sample were mixed with 10 mg of urea and 10 μl of isoelectric focusing sample buffer; 20 μl of this mixture were loaded onto the gel. Two-dimensional PAGE was performed as described previously[6]. The amount of LMP antigens was measured by densitometry of the resulting autoradiographs, and is reported in arbitrary units.

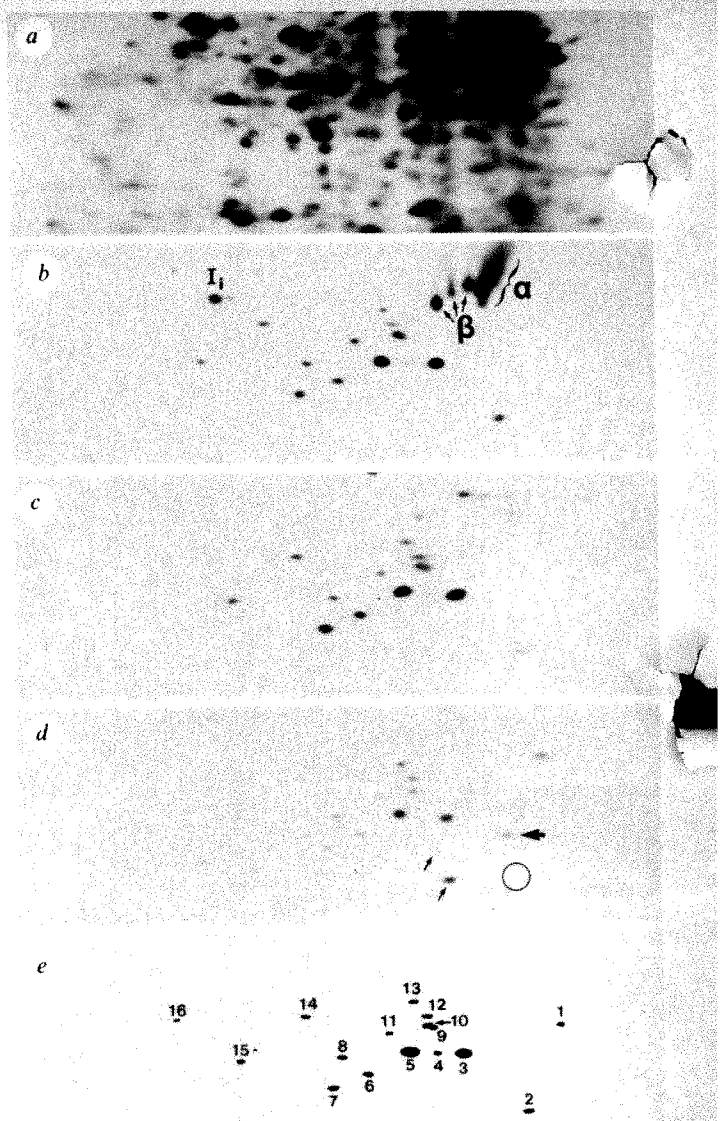


Fig. 2 Two-dimensional PAGE analysis of LMP antigens from unfractionated WEHI-3 extract (*a*), anti-H-2^d immunoprecipitate of WEHI-3 extract (*b*), peak LMP fraction (fraction 14, not immunoprecipitated) from Sepharose CL-6B column of WEHI-3 (*c*) and fraction 14 from the same column, but using IC21 extract instead of WEHI-3 (*d*). LMP chains are numbered in the schematic diagram (*e*) of the *H-2^d* haplotype pattern. Ia antigen α, β and invariant (I_i) chains are present in the immunoprecipitate (*b*) but not in the column fractions. The circle in *d* denotes the position of LMP chain 2, which seems to be absent from the *b* haplotype; the heavy arrow indicates its putative allelic form. Peptides denoted by the light arrows in *d* are probably not LMP chains, as they are also present in *H-2^k* haplotype column fractions (see Fig. 3*a*), but are not co-precipitated with the LMP complex from those cells (not shown).

the existence of a single LMP complex with a fixed stoichiometric ratio of subunits, rather than several types of complexes containing different ratios of the individual subunits and differing slightly in molecular size. However, it is formally possible that, in addition to the protein subunits already identified, non-^{35}S-methionine-labelled material (carbohydrates or peptidoglycans) are present in the LMP complex. In this respect, it may be significant that western blotting experiments have failed to reveal any such components (unpublished observations), indicating that if they do exist, they are probably not antigenic. When considered with the protein polymorphism data presented below, which indicate that only one of the LMP chains is polymorphic (and thus potentially antigenic) in the strain combinations used, these results argue strongly for a single complex containing all the LMP chains.

The antiserum we originally used to identify the LMP antigens was BALB.B (H-2^b) anti-BALB/c (H-2^d), which detects LMP antigens in the *a, d, f, ja, k, s, u, v* and *z* haplotypes. However, antisera made in the *d, k* or *s* haplotypes against the *H-2^b* haplotype fail to precipitate LMP-like polypeptides from *H-2^b* haplotype spleen cells or cell lines (J.J.M. & H.O.M., unpublished observations). In considering the possible function of the LMP antigens, it is important to decide between two possible explanations for this observation: (1) no LMP antigens are made in *b* haplotype mice or (2) although *b* haplotype mice synthesize LMP polypeptides, these are not immunogenic in *d, k* or *s* haplotype mice.

We can choose between these alternatives by using a non-immunochemical method of visualizing LMP antigens. Figure 2*d* shows the ^{35}S-labelled extract from the (H-2^b) IC21 cell line[9] manipulated in a manner identical to the WEHI-3 extract shown in Fig. 2*c*. At least 13 of the 16 LMP chains found in (H-2^d) WEHI-3 cells are also found in the IC21 cell line. LMP chains 4 and 16 are probably too weak to be seen in the IC21 gel (note that LMP chain 16 is also too weak to be seen in the immunoprecipitate shown in Fig. 2*b*). Thus, only one of the LMP chains found in WEHI-3 is clearly missing from the IC21 gel (LMP 2), and IC21 contains an extra chain which is not present in WEHI-3 (heavy arrow in Fig. 2*d*). Although definitive identification of the extra chain in the IC21 gel as part of the LMP complex will require either immunoprecipitation of the complex or peptide mapping/sequencing, this experiment strongly suggests that mice of the *H-2^b* haplotype produce a LMP complex similar to that in mice of other *H-2* haplotypes.

The above results are also consistent with the conclusion that the LMP are associated into a large complex; an apparent

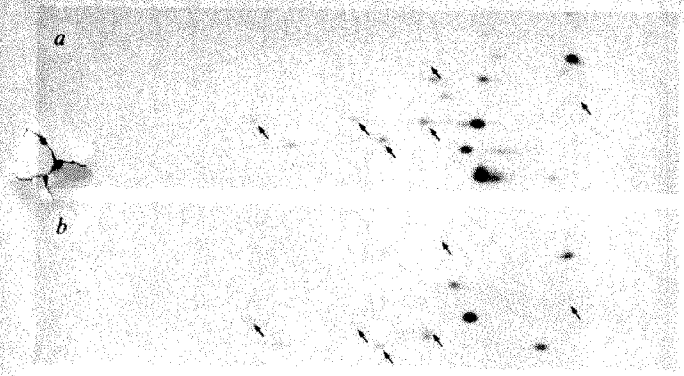


Fig. 3 Two-dimensional PAGE comparison of peak LMP column fractions (not immunoprecipitated) from the murine $H-2^k$ cell line UNC2 (*a*) and the human cell line, U937 (*b*). Chains which migrate to identical positions are denoted by arrows.

difference in only one of the peptides between the $H-2^b$ and $H-2^d$ haplotypes is sufficient to allow the production of an antibody which precipitates all 16.

As the gene products of the MHC have been highly conserved throughout evolution[5], one might expect that complexes similar to that described here would also be present in other species. The biochemical purification procedure described above might therefore allow the identification of such complexes in the absence of a specific precipitating antibody. Figure 3 shows a two-dimensional PAGE comparison of the peak LMP fraction from a murine cell line (UNC2, $H-2^k$)[10] and the corresponding fraction from the extract of a human cell line (U937)[11]. Although the patterns are not identical, the total number of chains present in this portion of the gel is similar, and at least six of the peptides from the human cell line migrate to exactly the same position as the corresponding murine LMP (arrows). It therefore seems likely that human cells do indeed contain a complex similar to the murine LMP complex. Definitive identification of these peptides as homologues of the murine LMP, and their genetic mapping, will, however, require further investigation.

Thus, both murine and human cells contain a large molecular complex composed of approximately 16 different polypeptide chains, all of which have a molecular weight of between 15,000 and 30,000. The unusual nature of this complex is highlighted by the fact that (non-immunoprecipitated) size-fractionated extracts can be made which, when electrophoresed in denaturing SDS gels, are seen to contain only LMP chains in the low-molecular weight portion of the gel. Thus, few or no other molecular species of similar size (M_r 500,000–650,000) contain any peptides of 15,000–30,000 M_r, whereas the LMP complex is composed entirely of subunits in this size range. (Although we have presented evidence elsewhere[6] that the multiple LMP chains are not simply proteolytic breakdown products of one or more larger molecule(s), we are currently attempting to verify this by characterizing the size of the messenger RNAs encoding the individual LMP chains.) In addition, excluding the ribosome (which has a molecular weight of $4–5 \times 10^6$ and may contain up to 80 different proteins[12]), we are unaware of any other biological macromolecule composed of so many different polypeptide chains. Thus, the LMP complex represents a unique macromolecular structure. That this unique structure is apparently conserved among species suggests that it is required for the proper functioning of the LMP complex. Experiments aimed at gaining insight into the nature of this function are underway.

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Role of γ-interferon in antibody-producing responses

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Interferon preparations, especially those containing γ-interferon (IFN-γ), have long been known to modulate immune responses[1–3]. However, because many studies used only partially purified interferons, it has been difficult to separate the immunoregulatory effects of the interferons from those of other biologically active molecules contaminating the preparations. Recently, with the cloning of the interferon genes in mouse and man, it has become possible to use these cloned interferons directly to test their effects in assays other than those involving the protection of cells from viruses. For example, cloned IFN-γ has been shown to be a potent inducer of Ia antigen expression on macrophages[4,5]. Similarly, cloned IFN-γ has been reported to act as a macrophage activation factors, as judged by the ability of activated macrophages to kill tumour cells *in vitro*[6]. We demonstrate here that cloned murine IFN-γ can also substitute for a late-acting helper factor which acts synergistically with other helper factors in the stimulation of B-cell antibody responses *in vitro*.

We have previously described[7] a murine T-cell hybridoma, FS7-20, which on stimulation with concanavalin A (Con A) produced at least five lymphokine activities: IFN-γ, macrophage activation factor (MAF), Ia-inducing factor (IaIF), a B-cell helper factor which we termed interleukin-X (IL-X), and interleukin-2 (IL-2). In studying a large panel of clones and subclones of FS7-20, we observed a random segregation of the ability to produce IL-2 from the ability to produce the other four lymphokine activities. On the other hand, IFN-γ MAF, IaIF and IL-X production were all tightly linked among these clones and subclones. We suggested that these four activities were probably due to the same molecular species, but we could not definitely rule out other possible explanations for the linkage or that some other common genetic control of production existed which varied among the clones and subclones of FS7-20. It had been suggested, and was later confirmed using cloned IFN-γ, that IFN-γ might act as IaIF and MAF[4–6,8], but an identity between IFN and IL-X was unexpected. Therefore, with the availability of cloned IFN-γ, we were particularly interested in testing its activity as a B-cell helper factor.

Our previous studies[9,10] had demonstrated that the optimal *in vitro* antibody responses of highly purified murine B cells to antigens such as sheep red blood cells (SRBC) required at least three different helper factors. The first was contained in preparations from normal macrophages or from the macrophage tumour cell line P388D1. The activity in these preparations has been indistinguishable from interleukin-1 (IL-1). The second factor preparation was the concentrated Con A-stimulated supernatant of the murine T-cell hybridoma, FS6-14.13 (ref. 11). Thus far the only known essential activity in this preparation has been IL-2 (refs 9, 10), although it remains possible that other as yet unidentified molecules in this supernatant contribute to

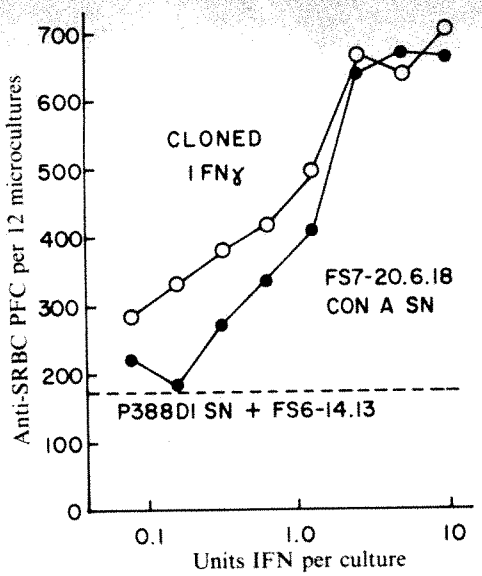


Fig. 1 IFN-γ is a B-cell helper factor. Data shown here are the sum of the anti-SRBC PFC obtained in 12 identical microculture wells of B cells incubated with P388D1 and FS6-14.13 supernatant (SN) clones (– – –) or with these supernatants and different numbers of units of IFN-γ contained in supernatants of the T-cell hybridoma FS6-20.6.18 (●) or derived from the cloned gene (○). **Methods:** Splenic B cells from (C57BL/6 × DBA/2)F_1 mice were purified by treatment *in vivo* with rabbit anti-mouse thymocyte serum (Microbiological Associates), and *in vitro* passage over Sephadex G-10 (Pharmacia) to remove macrophages, followed by treatment with a cocktail of anti-T-cell reagents and rabbit complement[9–11]. These B cells were incubated in 0.1-ml microcultures in limiting numbers (10^5 per well) with 0.005% SRBC and saturating concentrations of P388D1 supernatant (20%) as a source of IL-1 and 1 μl of a 50× concentrated supernatant from FS6-14.13 (containing 50 U μl IL-2) as a source of IL-2 and B-cell growth factor. 20 μl of supernatants containing different concentrations of IFN-γ were added at the optimal time, that is, after 24 h of culture. Supernatants were either dilutions of those induced by Con A treatment of the T-cell hybridoma FS7-20.6.18 (ref. 7), or dilutions of IFN-γ derived from the cloned gene[12,13]. The cloned gene was provided as a product purified from *E. coli* containing an expression vector that contained murine IFN-γ cDNA (specific antiviral activity of 8×10^6 U per mg protein)[12,13]. After 4 days' culture, wells were assayed for direct PFC against SRBC by standard techniques[9–11].

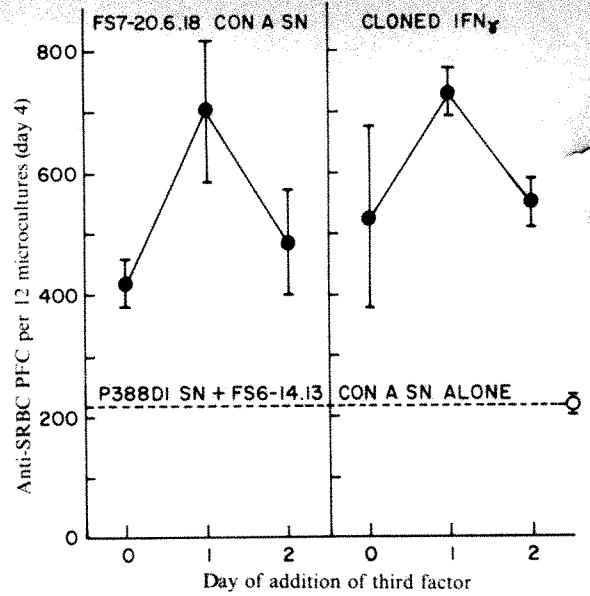


Fig. 2 IFN-γ is a late-acting helper factor. Cultures of purified B cells were set up as described in Fig. 1 legend with optimal concentrations of P388D1 and FS6-14.13 supernatants. Ten units of IFN-γ from either FS7-20.6.18 supernatant or from the cloned gene were added to the cultures on different days. After 4 days' total incubation the cultures were assayed for direct PFC against SRBC. Results shown are the total anti-SRBC PFC found in 12 identical microcultures.

its activity. The third required helper factor, which we termed IL-X because it did not correspond to any well-defined known molecule, was originally found in the 30,000–50,000 molecular weight fraction of an IL-2-depleted, Con A-stimulated supernatant of normal spleen cells. Its activity was assayed by its ability to enhance the antibody response of B cells to SRBC in cultures containing saturating amounts of IL-1 and IL-2 preparations. It was this activity which we later found in the Con A-stimulated supernatant of FS7-20 and whose production correlated strongly with that of IFN-γ.

To test directly whether IFN-γ could act as IL-X, we compared cloned IFN-γ produced in *Escherichia coli*[12,13] with a standard preparation of Con A-stimulated supernatant from FS7-20.6.18, a subclone of FS7-20 which produced high levels of IFN-γ and IL-X, but barely detectable levels of IL-2. Murine B cells were cultured for 4 days with SRBC, saturating amounts of IL-1 and IL-2 preparations and various concentrations of either cloned IFN-γ or the FS7-20.6.18 supernatant. Cultures were then assayed for anti-SRBC plaque-forming cells (PFC). The results are shown in Fig. 1. As we have reported previously[10], the combination of IL-1 and IL-2 stimulated a small anti-SRBC response from the B cells which was increased by addition of the FS7-20.6.18 supernatant (Fig. 1). The cloned IFN-γ produced a similar increase in the response (Fig. 1). For both preparations, the number of units of IFN-γ added predicted their relative activity in the PFC assay. Fifty per cent of the maximum stimulation was achieved with approximately one unit of IFN-γ per culture.

One of the properties distinguishing IL-X from IL-1 and IL-2 is that it can be added considerably later in the B-cell response. Whereas, to be fully effective, IL-1 and IL-2 must be added at the initiation of the response, the addition of IL-X can be delayed 1 or 2 days in a 4-day response[10]. To assess whether cloned IFN-γ behaved similarly, we again tested FS7-20.6.18 supernatant and cloned IFN-γ side by side in a kinetic experiment. B-cell cultures received SRBC, IL-1 and IL-2 on day 0 of a 4-day culture. On day 0, 1 or 2 the cultures received FS7-20.6.18 supernatant containing 10 U of IFN-γ or 10 U of cloned IFN-γ. Plaque responses were assayed on day 4. Stimulation of the response was seen again with both interferon preparations and as predicted, both preparations were active even when their addition was delayed 1–2 days, with day 1 being the optimal time of addition (Fig. 2). These results agree with our previous results using this activity isolated from normal T cells and distinguish the IFN-γ activity from that of IL-1 and IL-2.

Interferons have generally been reported as inhibitors of immune responses due to their anti-proliferative effects. However, there are several reports that interferon can be stimulatory, especially when added well into the proliferative phase of the response. In these studies, as in our own previous reports, unpurified interferon was used. The possibility that an associated non-interferon activity was responsible for the immunostimulation has always been a problem, especially when dealing with IFN-γ, which is produced from T cells and is therefore always contaminated with other lymphokine activities. In the present study, the use of IFN-γ produced from a recombinant DNA clone suggested that IFN-γ itself is stimulatory for B-cell antibody production.

In measuring B-cell helper factors *in vitro*, one must always consider the possibility that a particular factor may work indirectly by stimulating non-B cells contaminating the culture to produce additional activities. We have attempted to minimize this difficulty by developing and thoroughly testing B-cell purification techniques[9–11], and therefore believe it is highly likely that the IFN-γ activity in our experiments is due to its direct effect on the SRBC-responding B cell. This conclusion is

strengthened by the fact that despite an extensive search among normal T cells, hybridoma T cells and macrophage products, we have not identified an activity which can substitute for IFN-γ in this anti-SRBC B-cell response.

It is possible that some contaminant in the IFN-γ preparation from *E. coli*, for example lipopolysaccharide (LPS), is responsible for the effects on B-cell responses described here. The IFN-γ preparation we used is not mitogenic for B cells at concentrations containing 100 U ml^{-1} IFN-γ. Because effects on our cultures were seen at about 10 U ml^{-1} IFN-γ and because we have not seen similar effects with LPS at levels below 1 μg ml^{-1}, we consider this possibility unlikely. Moreover, the activity of our two IFN-γ-containing preparations (FS7-20.6.18 and cloned IFN-γ) is comparable in terms of units of IFN-γ. It is therefore highly unlikely that our results can be accounted for in any way other than that IFN-γ is responsible for the effects we measured.

Many laboratories have described T-cell-derived helper factors which stimulate B-cell antibody production. Such factors, which act late in the B-cell responses and do not seem to stimulate proliferation, have often been called T-cell replacing factor (or TRF) (reviewed in refs 15, 16). These activities have usually been defined in *in vitro* responses of murine B cells and are generally thought of as factors which stimulate the secretion of antibody from proliferating B cells. The question arises as to whether IFN-γ accounts for all T-cell-derived activities of this sort or whether other lymphokines can also provide this signal to B cells. As activities of this type have been reported in supernatants containing no detectable IFN-γ this is a distinct possibility. On the other hand, we have found that most of the late-acting TRF-like activity in the supernatants of mouse spleen cells stimulated with Con A, which presumably contain many if not all T-cell-derived lymphokines, has the properties of murine IFN-γ, that is, it has an apparent molecular weight of 40,000 on Sephadex gel filtration and is inactivated by incubation at *p*H 2. These findings suggest that IFN-γ is in fact a major component of TRF activities from normal lymphocytes. The lymphokine(s) responsible for driving the later events of B-cell maturation *in vivo* remains to be identified and will be the subject of future investigations.

Finally, one of the well known effects of IFN-γ which may be significant here is its inhibitory effects on cell proliferation. As IFN-γ is late acting, and in its capacity as a TRF is thought to stimulate differentiation of B cells to antibody secretion, this may be yet another example of a substance which inhibits cell division and simultaneously stimulates differentiation[14].

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γ-Interferon is one of several direct B cell-maturing lymphokines

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Two classes of molecules often released after the interaction of T lymphocytes, macrophages and antigen are B-cell maturation factors (BMF)[1–3] and immune (γ) interferon (IFN-γ)[4–7]. BMFs directly induce the maturation of resting B lymphocytes to the state of active immunoglobulin secretion[1–3], while IFN-γ is defined by the reduction of viral infectivity *in vitro*[8,9]. However, interferons have been shown to have a variety of effects[10–17] and they have also been reported both to increase and decrease B-cell differentiation in intact animals and complex cellular mixtures *in vitro*[18–25]. Here we show that murine IFN-γ produced by recombinant DNA technology[26] shows similar biological effects to BMFs from two other sources. All three preparations induce immunoglobulin secretion by both normal resting murine splenic B cells and the comparable B-cell tumour line WEHI-279.1 (refs 1, 3). IFN-γ and the other two BMFs are not identical, however, as anti-IFN-γ antibodies block the effects on B cells of IFN-γ, but not those of the other two lymphokines. IFN-γ may be one of several molecules with a direct role in driving the maturation of resting B cells to active immunoglobulin secretion.

We and others have recently begun to use inducible monoclonal cell lines, including tumours, as assay systems for lymphokines[1–3,27–29] as they overcome problems of interacting cell populations inherent in the use of more complex assay systems. Culture supernatants from the antigen-stimulated monoclonal helper T-cell line S26.5 (kindly provided by Dr M. Schreier)[1,30,31] are able to induce terminal differentiation to active IgM secretion in the B-cell tumour line WEHI-279.1 and also in comparable normal resting murine splenic B cells[1,3]. A second source of such maturational lymphokines is supernatant from cultures of spleen cells from mice homozygous for an autosomal recessive mutation known as viable motheaten (*me^v*). Viable motheaten homozygotes (*me^v*/*me^v*) show polyclonal immunoglobulin production (mostly of the IgM class) and autoimmune disease[32]. B-cell maturation in response to these lymphokines does not require interactions with other cells, occurs in most of the B cells present (under optimum culture conditions), is quite rapid (peak immunoglobulin secretion at days 2–3 of stimulation) and involves all of the pre- and post-translational alterations in IgM metabolism known to occur in the transition of normal resting B cells to immunoglobulin-secreting plasma cells[1,3]. The resulting immunoglobulin secretion has been measured by biosynthetic radiolabelling followed by immunoprecipitation, by enzyme-linked immunosorbent assays or, as done here, by a reverse plaque assay[33] which reveals the number of immunoglobulin-secreting cells in culture at the time of assay. To test directly the effects of IFN-γ in these B-cell maturation assays, we have used IFN-γ produced by CHO cells transfected[34] with a plasmid containing the cloned mouse IFN-γ gene[26]. This IFN-γ should be glycosylated and secreted similarly to that from normal T cells.

Culture supernatants from S26.5 T cells, *me^v* spleen cells and IFN-γ were titrated individually into cultures of either resting spleen cells from normal mice or WEHI-279.1 tumour B cells. The numbers of (immunoglobulin-secreting) plaque-forming cells (PFC) were determined 3 days later by the polyclonal reverse plaque assay[33]. As seen in Fig. 1, both normal (*a*) and tumour (*b*) B cells responded similarly to all three supernatants with an approximately 10-fold increase in PFC per culture. Cloned IFN-γ is clearly an effective BMF as measured in this

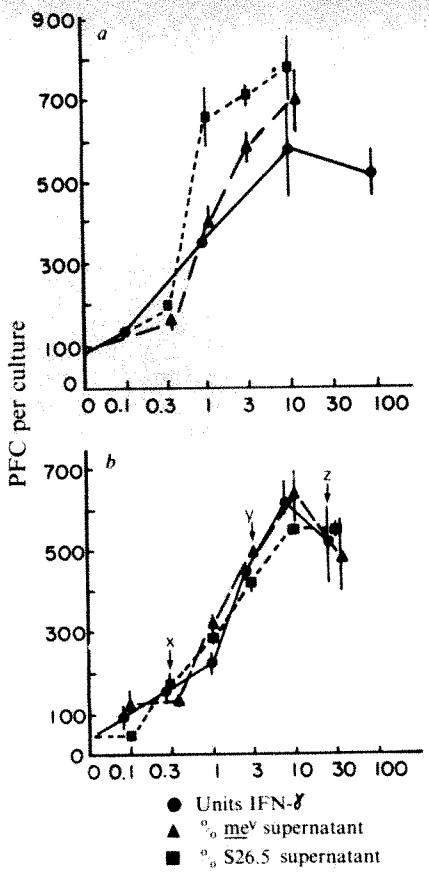


Fig. 1 Dose–response titrations of lymphokines from three sources. Antigen-stimulated S26.5 helper T-cell supernatant (■) (prepared as described[1]), non-stimulated *me*^{v} spleen cell supernatant (▲) (prepared by culturing *me*^{v} spleen cells at 10^7 per ml for 2 days) and supernatant from CHO cells transfected with plasmid containing the cloned murine IFN-γ gene (●) were tested on *a*, Percoll gradient-purified (that is, resting) C57BL/6J, DBA/2J or BALB/cBy spleen cells[1], or *b*, WEHI-279.1 (ref. 37) tumour B cells. Spleen cells were cultured in Iscove's modified Dulbecco's medium supplemented with albumin, transferrin and soybean lipids (IMDM-ATL)[38], while WEHI-279.1 cells were cultured in RPMI 1640-based medium containing 10% fetal bovine serum plus other supplements[3]. Cells were cultured at 10^3 per 0.2 ml flat-bottomed microwell at 37 °C in 5% CO_2/air, and the number of immunoglobulin-secreting cells (PFC) were determined on day 3 by the Protein A reverse plaque assay[33]. Percentages of the various supernatants, or amounts of IFN-γ activity were added to the cultures as shown. (The IFN-γ concentration in the undiluted CHO supernatant was 16,000 U ml^{-1}, as tested in an inhibition of cytopathic effect assay using encephalomyocarditis virus as the challenge virus and murine L929 cells as targets[9]. Data shown represent the mean ± s.e.m from two to four determinations each. Points x, y and z are referred to in Fig. 2 legend.

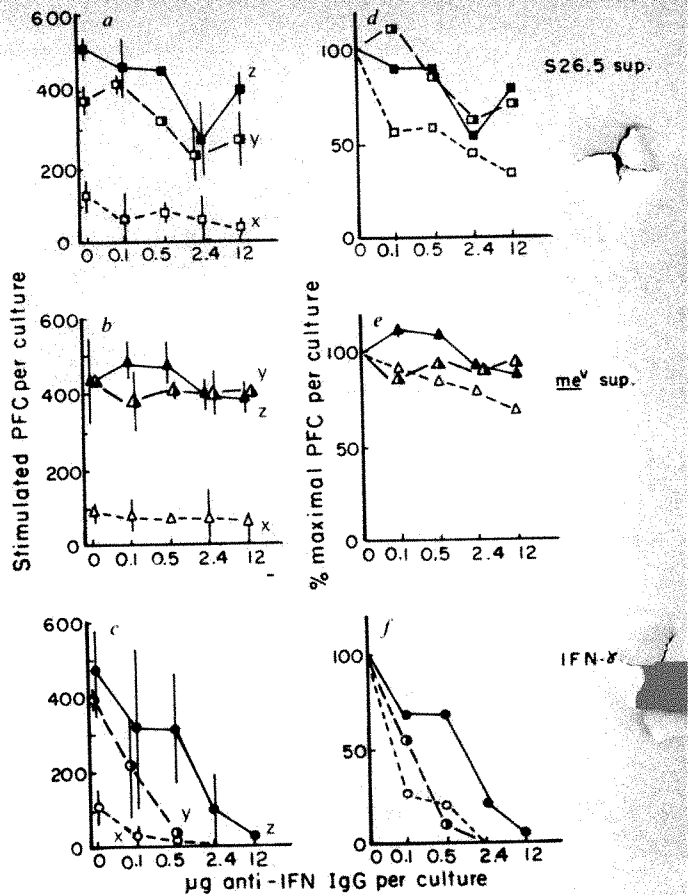


Fig. 2 Inhibition of lymphokine activities by rabbit anti-mouse IFN-γ antibodies. The indicated amounts of anti-IFN-γ IgG (capable of neutralizing 16 units of IFN-γ per μg) were added to the stimulatory lymphokine dose 30 min before WEHI-279.1 cells were added to the cultures. Culture conditions and harvesting were as described in the legend to Fig. 1. Panels *a*, *b* and *c* show stimulated PFC (that is, PFC over background), while panels *d*, *e* and *f* represent the same data expressed as the percentage of the response to that lymphokine concentration but without anti-IFN-γ IgG. *a*, *d*, Responses to S26.5 supernatant. *b*, *e*, Responses to *me*^{v} supernatant. *c*, *f*, Responses to IFN-γ supernatant. The curves indicated by open symbols and dotted lines refer to the minimum lymphokine concentrations indicated by x in Fig. 1*b*; the curves indicated by half-closed symbols and dashed lines refer to the near-maximal lymphokine concentrations indicated as y in Fig. 1*b*; and the curves indicated by closed symbols and solid lines refer to the super-optimal lymphokine concentrations indicated as z in Fig. 1*b*.

direct assay system. Control supernatants derived from CHO cells not transfected with IFN-γ-containing plasmids showed no PFC induction (data not shown).

When tested directly for inhibition of viral infectivity, S26.5 supernatant showed substantial IFN activity (500 U ml^{-1}), while *me*^{v} supernatant did not show detectable IFN levels (<10 U ml^{-1}). Therefore, we next tested whether anti-IFN-γ antibody (made in rabbits against partially-purified mitogen-induced mouse IFN-γ)[35] could inhibit the B-cell responses stimulated by the three different lymphokine preparations (Fig. 2). This antibody has been shown previously to block efficiently the anti-viral action of purified, naturally produced IFN-γ, but not to affect CSF, IL-1 and IL-2 (ref. 36). Varying amounts of IgG from the anti-IFN-γ antiserum were added to cultures containing WEHI-279.1 cells plus either barely stimulatory, almost maximally stimulatory, or supersaturating amounts of the three lymphokine preparations (doses labelled x, y and z respectively, on Fig. 1*b*). Fig. 2*a–c* shows the actual PFC responses seen in these cultures, while Fig. 2*d–f* shows the responses as percentages of the maximal response seen with that concentration of lymphokine but without anti-IFN-γ antibody. All doses of IFN-γ were completely inhibited by anti-IFN-γ antibody (Fig. 2*c* and *f*), with proportional amounts of anti-IFN-γ being required to inhibit different doses of IFN-γ. At the opposite extreme, no amount of anti-IFN-γ antibody tested produced a significant inhibition of the PFC response to any dose of lymphokine from *me*^{v} mice (Fig. 2*b* and *e*). The responses to supernatant from S26.5 T helper cells were partially inhibited, but no amount of anti-IFN-γ antibody used in these experiments was able to block completely the PFC response to even the lowest amount of S26.5 supernatant (Fig. 2*a* and *d*). Normal rabbit IgG was tested as a control for the anti-IFN-γ IgG and showed no inhibitory or stimulatory effects (data not shown). Rabbit (and murine) monoclonal antibodies to recombinant DNA-produced IFN-γ have also recently been tested, and shown to act like the rabbit antibodies to naturally-produced IFN-γ used here (data not shown).

To quantitate how much of the B-cell maturational activity in each lymphokine preparation might be due to IFN-γ, dose–response titrations were performed in the presence and absence

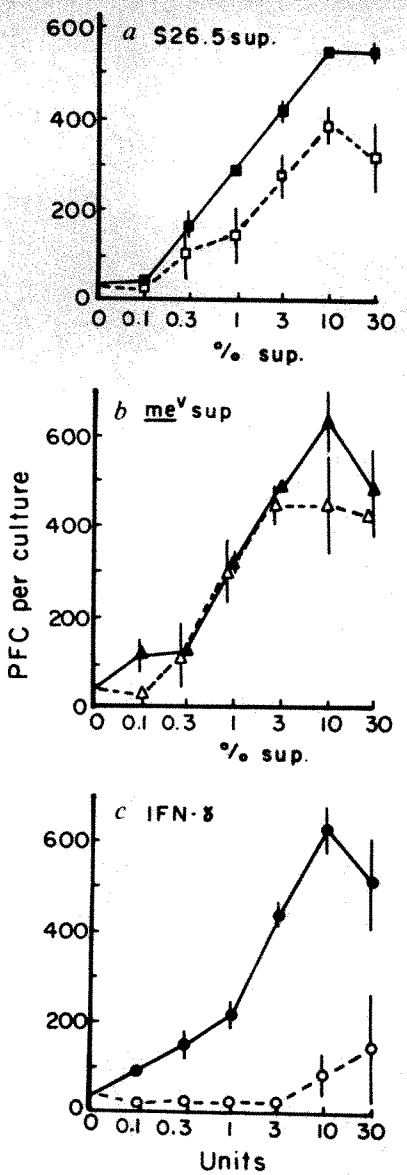


Fig. 3 Comparison of lymphokine titrations in the presence and absence of anti-IFN-γ antibody. The three lymphokine preparations were titrated into cultures of WEHI-279.1 cells as indicated in the legend to Fig. 1*b*. The solid symbols and lines indicate cultures with no anti-IFN-γ antibody, while the open symbols and dotted lines indicate cultures where 2.4 μg of anti-IFN-γ IgG (see Fig. 2—enough to block even the highest IFN-γ dose used here) were added to the lymphokines 30 min before the cells. *a*, S26.5 supernatant; *b*, *me*^{v} supernatant; and *c*, IFN-γ.

of sufficient anti-IFN-γ antibody to block a 10-fold superoptimal dose of IFN-γ (Fig. 3). As shown in Fig. 3*c*, the presence of this amount of anti-IFN-γ antibody shifted the dose–response curve for IFN-γ by about 100-fold, indicating that close to 100% of the activity in this supernatant is due to IFN-γ. The dose–response curve of the *me*^{v} supernatant (Fig. 3*b*) was not affected by the anti-IFN-γ antibody, suggesting that little (less than a few per cent) of the activity of this supernatant is due to IFN-γ. About half of the direct B-cell maturational activity in S26.5 supernatant appears to be due to IFN-γ, as this dose–response curve was shifted 2- to 3-fold by anti-IFN-γ (Fig. 3*a*).

From these experiments it can be concluded that IFN-γ functions as a direct B cell-maturing lymphokine, driving non-immunoglobulin-secreting normal and tumour B cells to active immunoglobulin secretion. In a representative experiment, 200 fluorescent-activated cell sorter (FACS)-purified normal resting BALB/cBy splenic B cells yielded 3 ± 1 PFC per culture on day 3 without added lymphokine, and 130 ± 14, 150 ± 14 and 130 ± 14 PFC with S26.5, *me*^{v} and IFN-γ supernatants, respectively. The comparable behaviour of monoclonal tumour B cells, normal resting splenocytes and FACS-purified resting B cells suggests that the induction of active immunoglobulin secretion occurs via the direct interaction of IFN-γ and the target B cells, without the required involvement of other cell types or signals. The effects of IFN-γ produced by recombinant DNA technology indicate that the active molecule is indeed IFN-γ, rather than another lymphokine which may also be found in naturally produced IFN-γ preparations. Previous studies have suggested both stimulatory and inhibitory effects of interferons on B-cell function, but always in the context of modulating the effect of another primary inductive signal (either antigen or mitogen) in complex cellular mixtures[10,18].

A second conclusion is that at least one, and probably two, other direct B cell-maturing lymphokines exist which are distinct from IFN-γ. These lymphokines may be related to IFN-γ, but do not show the anti-viral effects, nor the major antigenic determinant(s) seen by anti-IFN-γ antibodies, nor identical biochemical properties to IFN-γ. The non-IFN-γ BMF from S26.5 supernatant is less hydrophobic than IFN-γ—fractions 80–104 of the phenyl-Sepharose hydrophobic column shown in Fig. 3 of ref. 2 do not show anti-viral activity, and their BMF activity is not affected by anti-IFN-γ antibodies, whereas fractions 105–130 do show anti-viral activity and their BMF activity is completely blocked by anti-IFN-γ antibodies (data not shown). The *me*^{v} lymphokine is both smaller on gel filtration (molecular weight 15,000 compared with 50,000) and more acidic (*p*I 4 compared with 5.5) than IFN-γ (unpublished results). IFN-γ and the non-IFN-γ BMF molecules from S26.5 and *me*^{v} supernatants thus seem to be distinct and separable species.

Since IFN-γ is active in inducing the terminal maturation process which is central to the humoral immune response, and also influences the expression of specific macromolecules and cellular activities in other cell types[10–25], it is not clear whether the anti-viral activity for which IFN-γ was named represents its primary physiological function. In any case, the actual *in vivo* role of IFN-γ in humoral immune response induction, and its structural and functional relationships to the other molecule(s) with similar activity, will be important questions for future investigations.

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Cellular transformation by coordinated action of three peptide growth factors from human platelets

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Platelet-derived growth factor[1–4] (PDGF) is known to be involved in regulating the mitosis of connective tissue cells, and recent studies have also shown that it may function in mediating cellular transformation[5–8]. The oncogene carried by simian sarcoma virus, *sis*, is homologous to one chain of PDGF[5–8], and treatment of non-neoplastic cells with this growth factor results in increased transcription of another oncogene, *myc* (ref. 9). PDGF also stimulates the synthesis of proteins that are characteristic of transformed cells[10]. However, phenotypic transformation does not appear to result from the action of PDGF alone. For example, expression of *myc* does not transform cells in the absence of other oncogene expression[11]. We have recently shown that platelets contain another peptide growth factor, transforming growth factor-β (TGF-β)[12,13], in addition to PDGF. We report here that extracts of human platelets can induce anchorage-independent growth of non-neoplastic rat kidney (NRK) fibroblasts, but that purified PDGF alone does not elicit this effect. Rather, the transforming activity of the platelet extract is due to a concerted action of three distinct peptides: PDGF, TGF-β and a newly identified analogue of epidermal growth factor (EGF).

NRK fibroblasts are non-neoplastic, contact-inhibited cells that are indicators of phenotypic transformation. With the appropriate stimulus, these cells undergo anchorage-independent growth as assayed by colony formation in medium containing agar[14,15]. An acid–ethanol extract of human platelets induces phenotypic transformation of NRK fibroblasts (Fig. 1*a*). Homogeneous PDGF[16] does not induce anchorage-independent growth of the cells even when used at concentrations 10–20-fold greater than that required for half-maximal stimulation of DNA synthesis (Fig. 1*b*).

The results shown in Fig. 1 suggest that other components in the platelet extract, acting alone or in combination with PDGF, are responsible for the induction of phenotypic transformation. In fact, previous studies have shown that platelets contain a type-β transforming growth factor[12,13]. Figure 2*a* shows the elution position of this peptide during gel-filtration on a column of Bio-Gel P-60. TGF-β can induce anchorage-independent growth of NRK cells, but expression of this biological activity requires a synergistic interaction with EGF or a peptide resembling EGF (TGF-α)[12,17,18]. We can detect a new EGF-like peptide in human platelets by gel-filtration of the extract, adding purified TGF-β[12] to aliquots of the column fractions, and then assaying the samples for the ability to induce anchorage-independent growth (Fig. 2*b*). Column fractions 80–90 contain EGF-like activity as determined by synergism with TGF-β in the bioassay (Fig. 2*b*) and competition with ^{125}I-labelled EGF in a radioreceptor assay (Fig. 2*c*). Control studies showed that the biological activity of these fractions decreased at least 10-fold when assayed in the absence of TGF-β. The apparent molecular weight (M_r) of this platelet-derived, EGF-like peptide is 27,000

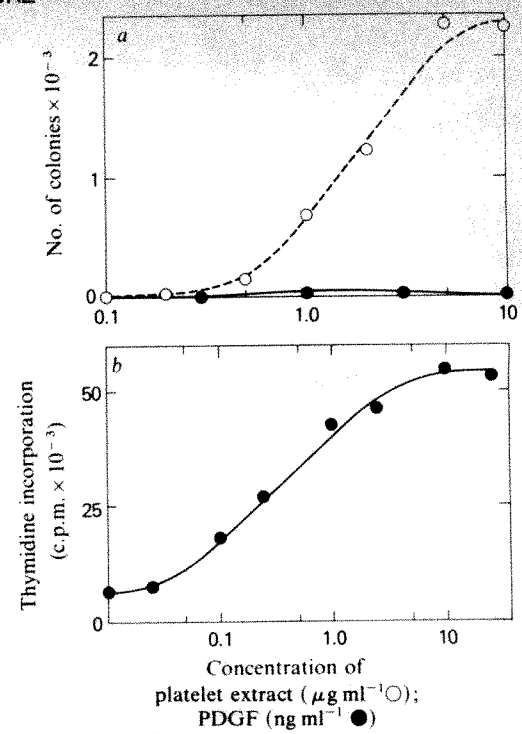


Fig. 1 Relative transforming activity of platelet extracts and PDGF. The acid–ethanol extract of human platelets was prepared and quantitated as described elsewhere[12]. *a*, The ability of the extract (○) or homogeneous PDGF[16] (●) to induce anchorage-independent growth of NRK fibroblasts as measured by the formation of colonies (>3,000 μm² per dish, ordinate) in soft agar. The concentrations of extract and PDGF (abscissa) are shown on logarithmic scale. This assay was performed in medium containing calf serum (10%, Gibco)[12,41]. *b*, The ability of purified PDGF to stimulate ^{3}H-thymidine incorporation into NRK fibroblasts (see ref. 12 for procedures).

as determined by gel-filtration (Fig. 2) and 24,000 as determined by preparative SDS-polyacrylamide gel electrophoresis[19]. (Fractions 84–88 were pooled, freeze-dried, and an aliquot of the residue was run on a 12.5% SDS gel. The gel was sliced, and eluted proteins[20] were assayed for the ability to induce anchorage-independent growth of NRK fibroblasts in the presence of TGF-β.) The EGF-like biological activity of this polypeptide shows that it is not the binding protein for EGF[21,22]. Its stability to both acidic *p*H (see Fig. 2 legend) and SDS-gel electrophoresis shows that it is distinct from the EGF-binding protein complex[21,22]. It was also possible that the 'new' peptide was PDGF, because PDGF, acting through its own receptor, can inhibit the binding of labelled EGF to the EGF receptor[23,24]. The EGF-like peptide identified in Fig. 2 is not PDGF; this new peptide competes with ^{125}I-labelled EGF in a radioreceptor assay using cells, line A431, that lack PDGF receptors[25–27] (Fig. 2*c*).

Oka and Orth[28] have recently suggested that human platelets contain both pro- and authentic EGF (M_r 33,000 and 6,000) as determined by reactivity in a human EGF immunoassay. The relationship between the larger peptide they detect and the EGF-like activity we detect using receptor binding competition (see Fig. 2) is not clear, but specific chemical modification studies indicate that the new growth factor described in Fig. 2 is intrinsically distinct from EGF (Fig. 3). Treatment of this platelet peptide with cyanogen bromide (CNBr) does not alter its affinity for the EGF receptor (Fig. 3*b*) despite the fact that: (1) amino acid analysis of the partially purified platelet peptide before and after CNBr treatment showed that cleavage at methionyl residues was complete, (2) EGF treated in parallel with CNBr showed the expected decrease[29,30] in receptor binding affinity (Fig. 3*a*), and (3) deliberate contamination of pure EGF with a large excess of protein (so that is was less pure than the preparation of the platelet-derived EGF-like peptide;

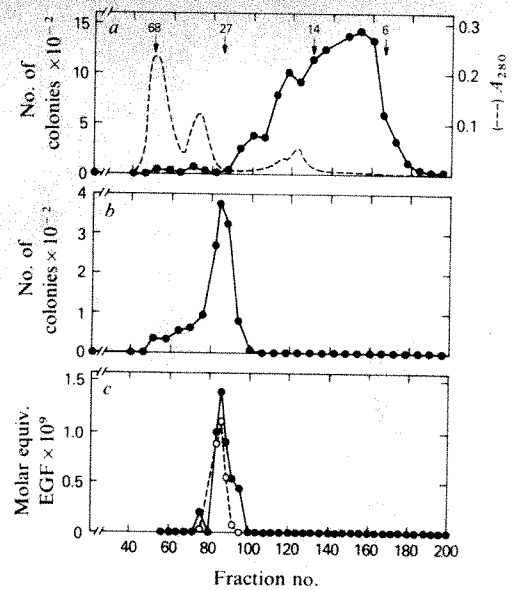


Fig. 2 Gel filtration of the human platelet extract on Bio-Gel P-60. *a*, Protein profile as determined by absorbance at 280 nm (– – –). Vertical arrows show, from left to right, the elution positions of bovine serum albumin ($M_r = 68{,}000$; V_0), chymotrypsinogen A ($M_r = 27{,}000$), lysozyme ($M_r = 14{,}000$) and insulin ($M_r = 6000$), respectively. ●, Elution position of platelet-derived TGF-β determined by colony formation (>9,000 μm^2) induced in the presence of pure murine EGF[42] (0.5 nM) and 2.5 μl of selected column fractions. *b*, Elution position of the platelet-derived, EGF-like peptide determined by colony formation (>9,000 μm^2) induced in the presence of homogeneous TGF-β (20 pM, prepared as described in ref. 12) and 250 μl of selected column fractions. *c*, Elution position of material that competes for the binding of ^{125}I-labelled EGF to the EGF receptor of NRK fibroblasts (●) and A431 cells (○).

Methods: The acid–ethanol extract of platelets (250 units) was solubilized in 1 M acetic acid (100 ml) and applied to a column (10 × 100 cm) of Bio-Gel P-60 which had been equilibrated in the sample solvent (1 M acetic acid, pH ~ 2). Fractions containing 35 ml were collected at a flow rate of 100 ml h^{-1}. The radioreceptor assay was performed similarly to the described procedure[34]. Cells were suspended (2×10^5 NRK cells ml^{-1} or 1×10^4 A431 cells ml^{-1}) in medium (Dulbecco's modified Eagle's medium with gentamycin) containing 10% fetal bovine serum (Bio-Fluids, Rockville, Maryland), seeded in 24-well plates (1 ml of the cell suspension per well), and incubated overnight (37 °C, humidified atmosphere of 10% CO_2 in air). Aliquots of selected column fractions (120 μl) were dried in the presence of bovine serum albumin (20 μg) in a vacuum desiccator. The residues were dissolved in 4 mM HCl (20 μl), diluted with binding buffer[34] (0.2 ml) containing ^{125}I-labelled EGF (about 2×10^5 c.p.m.), and incubated with washed cells as described previously[34]. Receptor binding competition data were converted to molar equivalents of EGF using pure murine EGF as the standard.

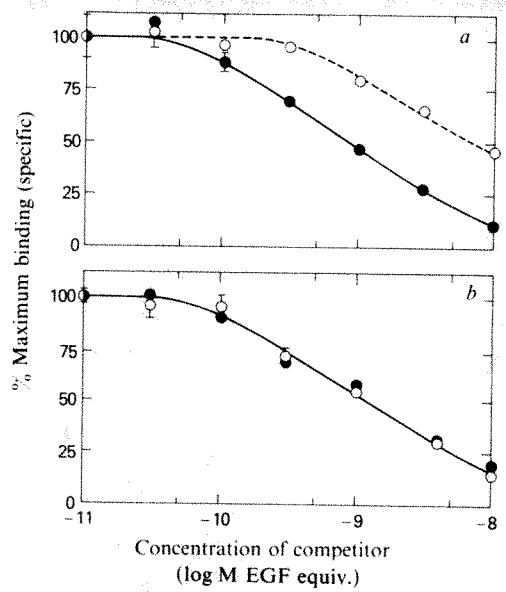


Fig. 3 The effect of CNBr on the receptor binding affinity of murine EGF (*a*) and the human platelet-derived EGF analogue (*b*). Results were obtained by measuring the ability of these peptides to compete with ^{125}I-labelled EGF for binding to the EGF receptor of NRK fibroblasts. The binding of labelled EGF to the cells was performed as described in Fig. 2 legend. Samples contained selected amounts of EGF or the platelet-derived EGF analogue, native (●) or after treatment with CNBr (○). Maximal binding of labelled EGF (determined in the absence of unlabelled competitor) was about 1,000 c.p.m.; nonspecific binding (determined in the presence of 10^{-7} M EGF) was about 150 c.p.m.

Methods: Treatment of EGF (0.5 nmol, 1 residue methionine per mol EGF[35,36,42,43]) and the partially purified EGF analogue (4×10^{-12} mol equivalents of EGF as determined by receptor binding competition; 0.5 nmol methionine in total protein as determined by amino acid analysis) with CNBr (0.25 μmol per sample) was performed in 70% formic acid (50 μl per sample) for 20 h at 23 °C. Native samples of EGF and the EGF analogue were treated in parallel in the absence of CNBr. After the reaction period, portions (10%) of the samples were removed for amino acid analysis. (Methionine values were decreased >90% by the CNBr treatment; other amino acids were not affected.) To ensure complete removal of CNBr and formic acid, the remainder of each sample was dried under a gentle stream of nitrogen (in the presence of bovine serum albumin, 100 μg), the residues were dissolved in 4 mM HCl (200 μl), and the resulting solutions were freeze-dried overnight. For the control studies, EGF (70 pmol) was deliberately contaminated with a large excess of protein (myoglobin, 60 nmol) before treatment with CNBr. The data are plotted as the mean of duplicate determinations using error bars to show the range (when observed).

see Fig. 3 legend) did not prevent its reaction with CNBr. Thus, the new EGF analogue in human platelets lacks a methionine residue critical to receptor binding.

Figure 4 shows the biological interactions between the three platelet-derived growth factors described above. In this experiment, cells were cultured in soft agar containing plasma-derived serum (rather than whole-blood derived serum) in order to decrease the basal levels of platelet peptides. Homogeneous PDGF in combination with either TGF-β alone or murine EGF alone does not efficiently induce phenotypic transformation (Fig. 4*a*). Similarly, TGF-β in conjunction with murine EGF can only minimally stimulate anchorage-independent growth in this system if the concentration of added PDGF is less than 0.1 ng ml^{-1} (Fig. 4*a*). When platelet-derived peptides are absent from the basal assay medium, phenotypic transformation requires the addition of all three growth factors (Fig. 4*a*). In three separate experiments, PDGF (3 ng ml^{-1}) 'restored' the activity of TGF-β (20 pM) and murine EGF (0.5 nM) to 50, 100 and 65% of that observed with autologous 10% whole-blood derived serum. Furthermore, Fig. 4*b* shows that in a plasma-derived system, the human platelet-derived EGF analogue efficiently synergizes with PDGF and TGF-β to induce transformation.

Recent studies have emphasized the relationships between PDGF and oncogenesis[5–10]. The results described here show that PDGF alone does not induce transformation in NRK cells, but that it acts in concert with platelet-derived TGF-β and EGF-like peptides. These data agree well with previous studies indicating that PDGF controls proliferation by modulating the response of cells to other growth factors[31–33]. Mechanistically, TGF-β can increase the number of cell-surface receptors for EGF and alter their down-regulation in response to ligand binding[34]. The platelet-derived EGF analogue can bind to these receptors (Figs 2, 3) and exert EGF-like effects (Fig. 4). This

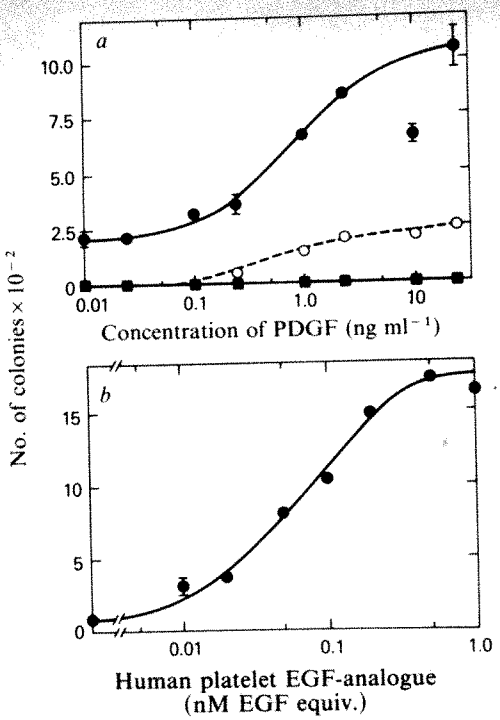


Fig. 4 Biological interaction of the three platelet-derived growth factors. The amount of PDGF in the samples was varied (abscissa, logarithmic scale). The concentrations of TGF-β and EGF were constant at 20 pM and 0.5 nM, respectively. *a*, Colony-forming activity of NRK fibroblasts (colonies >3,000 μm^2) in the presence of PDGF, TGF-β and EGF (●); PDGF and TGF-β (■); or PDGF and EGF (○). *b*, The ability of the human platelet-derived EGF analogue to induce colony formation of NRK cells in soft agar when assayed in medium containing 10% plasma-derived serum, TGF-β (20 pM) and PDGF (3 ng ml^{-1}).
Methods: The assay was performed as before[12,41] except that the medium contained 10% plasma-derived serum (bovine) rather than 10% calf (whole blood-derived) serum. TGF-β, PDGF and murine EGF were purified to homogeneity[12,16,42]. The platelet-derived EGF analogue was partially purified by gel filtration (see Fig. 2) and HPLC on a C_{18} column (Vydac, semipreparative) using 0.1% trifluoroacetic acid (TFA) as the stationary phase and 0.1% TFA in an acetonitrile gradient as the mobile phase (10–40% acetonitrile in 3 h, 0.8 ml min^{-1}). Fractions containing 0.8 ml were collected. The elution position of the EGF analogue (about 15% acetonitrile) and its quantitation were determined by assaying aliquots of column fractions in the radioreceptor assay with A431 cells (see Fig. 2 legend for details). Plasma-derived serum (10% in medium) was prepared by adding fresh bovine plasma (11 ml) to medium (100 ml of Dulbecco's modified Eagle's medium containing gentamycin). A fibrin clot formed and retracted during overnight incubation at 37 °C. After removal of the clot, the resulting solution was sterilized by filtration. Autologous bovine whole-blood derived serum (10% in medium) was prepared similarly using the serum from freshly clotted whole blood. The plasma and serum were derived from the same bleeding.

new growth factor is structurally distinct from authentic EGF as judged by its apparent larger molecular weight and by the absence of a methionine residue critical to receptor binding (the methionine at position 21 in both human[35] and murine[36] EGF). In the latter regard, this analogue resembles the type α transforming growth factors[15,17] which are EGF-like peptides lacking methionine[37,38]. Similarly, this critical methionine residue is absent from the potential EGF-related peptides encoded in the mouse EGF mRNA[39,40]. Our results show that the human platelet contains at least three distinct growth factors and that, in the presence of plasma, the interaction of these three peptides can induce transformation of fibroblastic cells.

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Human epidermal growth factor receptor cDNA is homologous to a variety of RNAs overproduced in A431 carcinoma cells

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The recently discovered similarity between the human epidermal growth factor (EGF) receptor and the avian erythroblastosis virus v-*erb-B* protein[1] supports the hypothesis that viral oncogenes share a common evolutionary origin with genes encoding growth-regulating cell-surface receptors. To elucidate the relationship between receptors and malignant transformation, we have now used a fragment of v-*erb-B* as a probe to screen a cDNA library of mRNA from A431 human carcinoma cells, which possess a large number of EGF receptors[2]. Of the six clones isolated, the largest (pE7)

contains an insert of 2.4 kilobase pairs (kbp) whose deduced amino acid sequence is homologous to the v-*erb-B* protein and identical to reported EGF receptor peptide sequences[1]. This pE7 cDNA hybridized to three prominent RNAs of ~10, 5.6 and 2.9 kilobases (kb), and to three minor species of 6.3, 4.6 and 3.3 kb. All were present in elevated levels in A431 cells. The prominent 2.9-kb RNA was homologous only to the 5′ portion of the pE7 insert. This result raises the possibility that differential RNA processing is used by A431 cells to generate a variety of RNAs.

EGF elicits a wide variety of rapid and delayed responses mediated via specific high-affinity cell-surface receptors[3]. These responses to EGF are dependent on the occupation of functional receptors at the cell surface. Short-term effects of EGF include activation of transport systems[4–6] and increased protein phosphorylation[7]. Delayed responses include stimulation of DNA, RNA and protein synthesis that ultimately results in cell proliferation[8–10].

The EGF receptor is a glycoprotein of molecular weight 170,000 which has been identified in many types of cells[11–15]. The receptor has an EGF-stimulated protein kinase activity, which phosphorylates itself and other substrates on a tryosine residue[7]. Regulation of the cellular response to EGF can occur through ligand-induced 'down regulation' of the receptor[16–20].

Recently, six different peptides from the EGF receptor have been isolated, sequenced and found to be strikingly similar to the deduced amino acid sequence of v-*erb-B*[1]. The latter is the protein product of the *erb-B* gene of avian erythroblastosis virus (AEV) which induces both erythroblastosis and sarcomas in infected birds[21,22]. Most mammalian cells contain homologous counterparts to the viral transforming genes[23,24], whose enhanced expression is thought to produce the malignant phenotype[25–27]. The strong similarity in protein structure between v-*erb-B* and the EGF receptor supports the idea that the human cellular homologue to v-*erb-B* (c-*erb-B*) may be the same gene as that coding for the EGF receptor. This hypothesis is reinforced by the finding that the c-*erb-B* gene and the EGF receptor gene are both localized on the same region of human chromosome 7 (refs 28, 29).

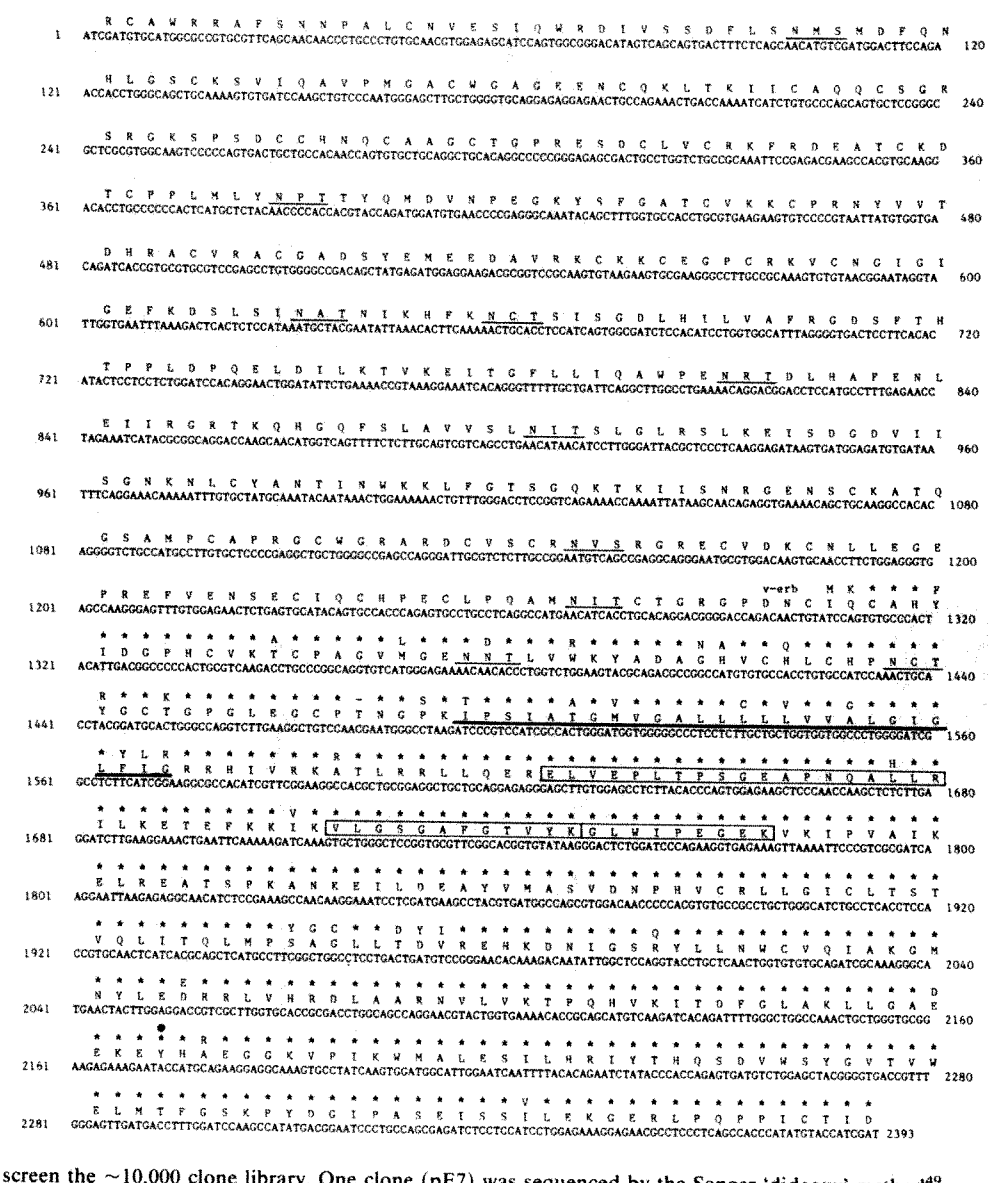


Fig. 1 Nucleotide sequence and deduced translation of the pE7 cDNA insert. The nucleotide sequence of the coding strand of pE7 is presented with the 5′-terminal *Cla*I site as position number 1. The amino acid sequence, represented by single-letter abbreviations, is shown directly above the DNA sequence. The translation of the v-*erb-B* (ref. 30) gene product is aligned with the pE7 translation and presented above it, beginning at nucleotide 1,305. Identical predicted amino acid sequences are indicated by asterisks (*). To optimize the similarities between two sequences, a nucleotide triplet has been removed at position 1,476, indicated by (—). Lightly underlined amino acid triplets represent potential *N*-linkage glycosylation sites, heavily underlined sequences indicate the location of the likely transmembrane region of the protein. A dot (•) points to the putative kinase phosphoacceptor[7,44]. Boxed sequences represent those amino acids shared with published EGF receptor peptide sequences[1]. The nucleotide sequence corresponding to the v-*erb-B* *Bam*HI fragment used to screen the library is located at 1,756–2,314.

Methods: Poly(A)$^+$ RNA was isolated from total A431 RNA[45] and used as a template for AMV reverse transcriptase synthesis of cDNA using oligo(dT) as primer[46,47]. After second strand synthesis[47], the cDNA was treated with S_1 nuclease (1 U μl^{-1}), blunt-end ligated to *Cla*I artificial linkers, restricted with *Cla*I, and after fractionation over Sepharose 4B to remove cDNA <500 bp, the cDNA was ligated into *Cla*I-digested pBR322 and cloned into *Escherichia coli* N38. A 0.5-kbp *Bam*HI v-*erb-B* fragment[30,31] was nick-translated[48] and used as probe to screen the ~10,000 clone library. One clone (pE7) was sequenced by the Sanger 'dideoxy' method[49]. The 2.4-kb insert was excised from pBR322 by restriction endonuclease cleavage with *Cla*I and purified by preparative electrophoresis on low melting point 1.0% agarose gels[50]. After further digestion with selected restriction endonucleases, fragments of the cDNA insert were ligated into the appropriate molecular cloning sites of bacteriophage M13 substrains mp-8–9, or –19 (ref. 51). After transfection into *E. coli* strain JM-101, single-stranded recombinant phage DNAs were isolated[52]. All DNA sequences were obtained by the dideoxy nucleotide chain termination method[49,52]. The single-stranded subclones were selected first at random and later by hybridization to overlapping fragments cloned in bacteriophage M13 in the opposite orientation. All M13 inserts were sequenced using a flanking universal primer[53]. The complete contiguous sequence was assembled from individual, overlapping subclones containing fragments in either orientation, using the programs described by Staden[54] modified to run on an IBM-3081 computer.

Here we report the isolation and characterization of cDNA clones obtained from an A431 cDNA library using a v-*erb-B* probe from the '*src* family' region that is known to be conserved among oncogenes[30,31]. The plasmid pEB1 containing the v-*erb-B* sequence was given by Dr Tadashi Yamamoto (University of Tokyo). Double-stranded cDNA synthesized using A431 poly(A)$^+$ RNA as template, was ligated to *Cla*I linkers and inserted into the *Cla*I site of pBR322. Out of the ~10,000 clones screened, six hybridized to a 0.5-kbp v-*erb-B* *Bam*HI fragment (pE3, 4, 5, 6, 7, 8). One of these clones (pE7) had a 2.4-kbp DNA insert flanked at both ends by *Cla*I restriction sites. A second (pE3) contained a 1.3-kbp DNA *Cla*I insert. Homology between pE7 and the other cloned cDNAs was demonstrated by hybridizing ^{32}P-labelled pE7 insert DNA to other blotted plasmid DNAs.

The sequence of the 2.4-kbp pE7 insert (Fig. 1) contains one open reading frame which converts to ~790 amino acids; the carboxy-terminus exhibits great homology to the v-*erb-B* protein sequence (Fig. 1), and limited similarity to RSV p60^{src} (ref. 30). In addition, three of the six EGF receptor peptides sequenced by Downward *et al.*[1] match exactly with the deduced amino acid sequence of pE7 (Fig. 1, boxed sequences). The other three are not contained in the 2.4-kbp insert because during the cloning of this DNA a natural *Cla*I site within the original cDNA molecule (now representing the 3′ end of the pE7 insert) was cleaved by *Cla*I before ligation into pBR322, thereby removing the extreme 3′ portion of the cDNA.

It is not yet clear whether the c-*erb-B* gene product and the EGF receptor are identical or simply related proteins. In principle, this pE7 cDNA clone could be derived from mRNAs encoding either the human c-*erb-B* gene product or the EGF receptor. We believe that pE7 codes for the EGF receptor because of the structural similarity between the pE7 deduced amino acid sequence and the EGF receptor peptides, and because RNA levels quantified by hybridization with pE7 correlate extremely well with EGF receptor protein levels in a variety of cell types (Y.-h.X. *et al.*, in preparation).

An interesting feature of the pE7 sequence is a hydrophobic stretch of 27 amino acids (Fig. 1, darkly underlined) of which 20 are nonpolar and 7 are neutral; this is probably the hydrophobic transmembrane region of the EGF receptor. The putative site for a phosphoacceptor is shown in the sequence (Fig. 1, tyrosine •). In addition, there are ten potential *N*-glycosidic linkage sites (Asn-Met-Ser; Asn-Pro-Thr; Asn-Ala-Thr; Asn-Cys-Thr; Asn-Arg-Thr; Asn-Ile-Thr; Asn-Val-Ser; Asn-Ile-Thr; Asn-Asn-Thr; Asn-Cys-Thr; Fig. 1, lightly underlined)[32,33] which could be involved in the complex glycosylation that occurs on the extracellular portion of the EGF receptor[34,35]. Mayes and Waterfield[36] have reported that biosynthesis of the human EGF receptor in A431 involves co-translational addition of at least seven *N*-linked oligosaccharide chains. It is possible that most or all of the potential sites derived from this cDNA sequence are used in EGF receptor processing.

The fact that there exists one full-length open reading frame suggests that this cDNA was synthesized from a single mRNA molecule, and not formed from a recombinational event between two different cDNAs in the same vector. The ability of the insert to be cut out of the vector cleanly by *Cla*I also supports the likelihood of a normal recombinant plasmid construction. To prove that this long cDNA was made from a single mRNA, R-looping was performed using *Sal*I-linearized pE7 (no sites in the insert) and A431 mRNA. Analysis of numerous R-loops revealed that the extreme 5′ end of the cDNA insert was consistently associated with RNA (long tail of pBR322); however, some variability in RNA hybridization was detected at the 3′ end (short tail of pBR322). The data suggest that at least two categories of RNA–DNA hybrids exist (see Fig. 2 legend). One type of RNA forms a full-length hybrid, with colinear homology over the entire span of the insert (Fig. 2*A*). Many of these full-length RNA–DNA structures have RNA tails at both ends, suggesting that a significantly longer RNA is responsible for the R-loop formation. This RNA probably

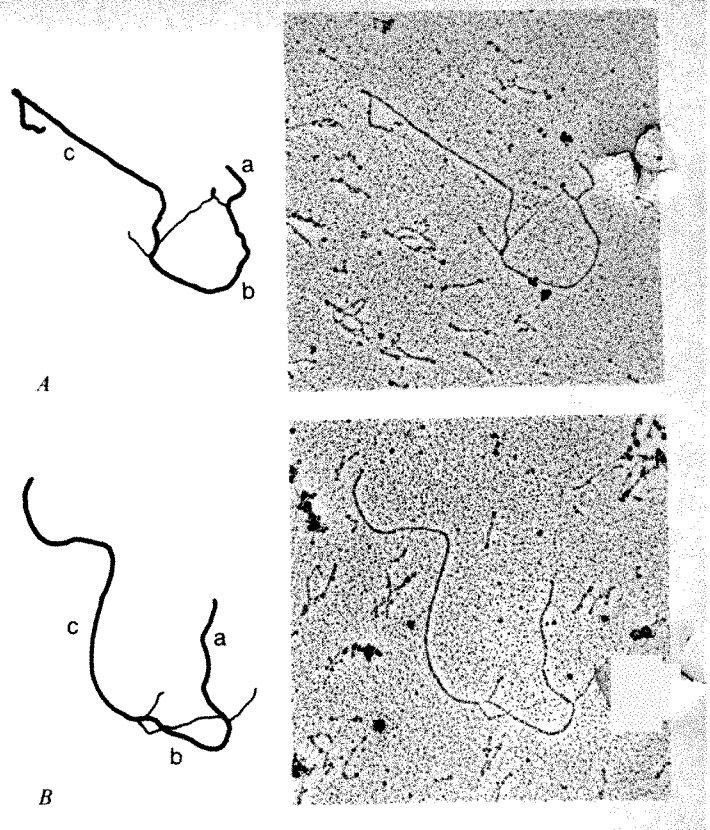


Fig. 2 R-loop analysis of pE7 using A431 poly(A)$^+$ RNA. Purified plasmid pE7 was linearized by *Sal*I digestion (cuts once in pE7 within the pBR322 portion), and hybridized with poly(A)$^+$ RNA. The hybrid molecules were spread and analysed[55]. a, Short arm of pBR322; b, RNA–DNA hybrid; c, long arm of pBR322. The thin strand in the b region represents the noncomplementary single-strand insert DNA. *A*, *B*, Representative molecules which fall into two classes of R-loops. *A*, The average size and standard deviation (in bp) of the sections represented by a, b and c are 646 ± 223, 1,673 ± 250, and 3,570 ± 415, respectively. Although only a 5′ RNA tail is seen in this R-loop, other R-loops showed tails at either the 3′ end, the 5′ end, or both. *B*, The average sizes are 1,312 ± 99, 1,293 ± 171 and 3,583 ± 294. ×16,000.

represents the normal EGF receptor mRNA, which would contain the coding region homologous with previously sequenced EGF receptor peptides, and which would have additional RNA regions extending beyond the cDNA insert at both ends of the molecule.

A second RNA forms an R-loop that is ~600 nucleotides shorter at the 3′ end (Fig. 2*B*). This structure, which also appears to have tails at both ends, is seen more often than the longer R-loop described above. The frequent appearance of the shorter RNA–DNA hybrid molecule suggests that a second RNA exists in A431 cells which has strong homology to the 5′ end of the pE7 insert, but not to the 3′ end.

If the 5′ end of the pE7 insert alone was capable of hybridizing to a different species of RNA, as suggested by the R-loop data, then Northern analysis using 3′- and 5′-specific pE7 cDNA fragments should confirm this. Messenger RNA was isolated from A431 and A498 cells (a human kidney carcinoma cell line which has about one-third the level of EGF receptors as A431; N. Richert and I.P., unpublished data), electrophoretically fractionated on 1% agarose, transferred to nitrocellulose, and hybridized to various cDNA probes. Figure 3 (lanes *a*) shows that the nick-translated 0.5-kbp *Bam*HI v-*erb-B* probe used to screen the cDNA library hybridizes to two prominent A431 RNAs of ~10 kb and 5.6 kb. Hybridization to A498 RNA gave a 4–5-fold less intense signal (Fig. 3*b*). Hybridization of the human pE7 probe to A431 RNA blots revealed a variety of

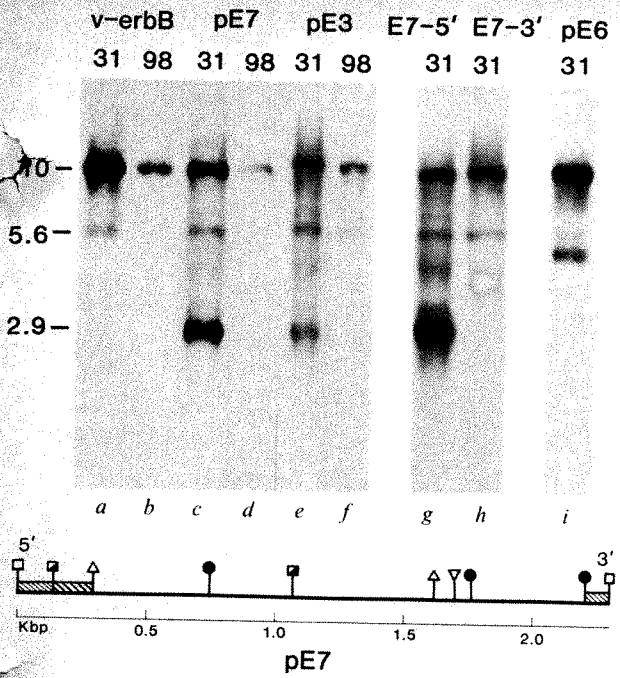


Fig. 3 Northern analysis of EGF receptor RNA. Poly(A)$^+$ RNA was isolated as described in Fig. 1 legend, either from A431 cells (31, lanes *a*, *c*, *e*, *g–i*) or A498 cells (98, lanes *b*, *d*, *f*), fractionated on 1% agarose, and transferred to nitrocellulose[56]. Nick-translated probes include the 0.5-kbp *Bam*HI v-*erb-B* fragment (lanes *a*, *b*);the 2.4-kbp cDNA insert from pE7 (lanes *c*, *d*); the 1.3-kbp insert of pE3 (lanes *e*, *f*); the 5′-specific *Cla*I-*Pst*I pE7 fragment (lane g); the 3′-specific *Bam*HI–*Cla*I pE7 fragment (lane *h*); and the 600-bp pE6 insert (lane *i*). Hybridization was in 50% formamide, 5×SSC at 42 °C for 36 h. Washing included 1 h in 0.2× SCC, 0.1% SDS at 45 °C. Numbers at the left indicate the size of the major RNA species in kilobases. Sizes were determined using rRNAs as markers, and the size of the 10 kb species is therefore an estimation. The restriction endonuclease map of the EGF receptor cDNA insert of pE7 is shown at the bottom. The region of pE7 corresponding to the v-*erb-B* 0.5-kbp probe is located approximately at the 1.75–2.25-kbp *Bam*HI–*Bam*HI fragment (see Fig. 1). The pE3 insert is located from ~1.0–2.3 kbp and the pE6 insert from 1.8 to 2.4 kbp on the map of pE7. The locations of fragments used for 5′- and 3′-specific hybridization are indicated by lined boxes. Restriction enzymes include *Cla*I (□), *Eco*RI (▽), *Pst*I (△), *Bam*HI (●) and *Pvu*II (◪). A more detailed restriction map is available from the authors on request.

RNAs, including the 10-kb and 5.6-kb species (Fig. 3*c*). In addition, three minor RNAs (6.3, 4.6 and 3.3 kb) and a prominent 2.9-kb species were found in the A431 cells. These RNAs were all present in elevated levels in A431 cells relative to A498 cells (Fig. 3*c*, *d*). This is consistent with the possibility that many, if not all, of the RNAs are related to the EGF receptor. It seems likely that these RNAs are generated by either complex splicing, or are transcribed from a gene family with multiple members localized on several chromosomes in A431 cells.

When the 1.3-kbp insert of pE3 was used as probe, the same RNAs were found (Fig. 3*e*, *f*). However, hybridization with a pE6 probe which contains a small ~600-bp cDNA insert homologous to the 3′ end of pE7 failed to visualize the 2.9- and 3.3-kb RNA species in A431 cells (Fig. 3*i*). The same result was obtained when a 90-bp fragment from the 3′ portion of the pE7 was used as a probe to hybridize to A431 RNA blots (Fig. 3*h*). However, a 290-bp fragment from the 5′ portion of pE7 hybridized to all the species detected by full-length pE7, including the 2.9- and 3.3-kb RNAs (Fig. 3*g*). The smaller 2.9-kb RNA is a relatively prominent species in A431 cells and is probably the species exhibiting great homology to ~1.5–1.6 kbp of the 5′ portion of the pE7 insert (R-loop analysis, Fig. 2*B*); it therefore could code for a truncated form of the EGF receptor. Recently, it has been reported that A431 cells secrete a truncated EGF receptor-like protein of the molecular weight 95,000–105,000 (refs 36, 37). This protein could be translated from a small A431 RNA (2.9 or 3.3 kb RNA). A 16-amino acid clostripain fragment of this truncated protein recently sequenced by Weber *et al.*[37] matches exactly with a stretch of amino acids deduced from clone pE7 at nucleotide positions 926–974 (Fig. 1). This region of the cDNA is homologous to the small RNA species as well as the large RNA (Figs 2, 3). These data confirm the close relationship between the truncated form and the full-length EGF receptor, and is consistent with the notion that one or both of the small RNAs encode the short receptor-like protein.

We recently reported that DNA sequences for the EGF receptor were amplified 30-fold and possibly rearranged in A431 cells[38]. Shimizu *et al.*[39] have reported that in addition to two normal chromosomes 7 that contain the EGF receptor gene, A431 cells contain two marker chromosomes (M4 and M14) originating from translocations between chromosome 7 and unidentified segments of DNA. One of these (M4) confers high EGF-binding capacity to fused A431/mouse A9 cells. These data raise the possibility that the 10-kb and/or the 5.6-kb RNA are transcribed from a normal EGF receptor gene on chromosome 7, and the 2.9-kb RNA species is transcribed from a chromosome involved in a rearrangement event at or near the location of the EGF receptor gene in A431 cells (possibly M4 and/or M14). However, a new RNA species could be generated by any gene disruption causing alterations in the normal start site, stop site, or patterns of RNA splicing. Whether or not this RNA encodes a protein which is responsible for the elevated EGF-binding ability of A431 cells remains to be established.

An additional interesting finding concerns heterogeneity between the different cDNA clones, as determined by restriction mapping and partial sequence analysis (data not shown). Further work is needed to determine whether this represents differential RNA splicing of an unprocessed EGF receptor gene product.

One striking characteristic of the EGF receptor gene is its similarity to the v-*erb-B* gene[1]. Because of the apparent functional relationship between growth factor receptors and oncogene products, it is interesting to compare our results for the EGF receptor with those involving other oncogene proteins. Transformed cells expressing high levels of either *myc* or *myb* protein were found to transcribe RNAs of unusual size, and to contain chromosomes involved in translocation events[40–43]. One possible explanation is that the overproduction of the 2.9-kb RNA and its corresponding modified protein product may be associated with the appearance of the malignant phenotype of A431 cells. To address this question, and to elucidate the nature of a putative EGF receptor gene rearrangement in A431 cells, we are now characterizing cDNA clones encoding the 2.9-kb RNA.

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Note added in proof: Since submitting this paper, two reports[57,58] reaching essentially the same conclusions, have been published.

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Generation of β-globin by sequence-specific proteolysis of a hybrid protein produced in *Escherichia coli*

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High-level expression of many eukaryotic genes has proved difficult to achieve even when a strong promoter[1–3] and the ribosome binding sequence[4,5] from highly expressed *Escherichia coli* genes have been placed in front of the coding sequences. To overcome this problem, many eukaryotic proteins have been efficiently produced as hybrids after fusion of their genes with a coding sequence of *E. coli* genes[6]. However, such hybrid proteins are not suitable for functional studies or clinical use unless the authentic protein sequence can be released by specific cleavage. Here, we have inserted the sequence Ile-Glu-Gly-Arg between the 31 amino-terminal residues of λ *c*II protein and Val 1 of human β-globin, and produced this hybrid in high yield in *E. coli*. We then cleaved the hybrid specifically at the single arginine, using blood coagulation factor X_a and thus liberated the authentic β-globin chain. As factor X_a is specific for the tetrapeptide Ile-Glu-Gly-Arg[7], which is rare in protein sequences, our expression/cleavage system is applicable to the efficient production of many eukaryotic proteins.

Table 1 Peptide bonds known to be cleaved by blood coagulation factor X_a

P_4	P_3	P_2	P_1		P'_1	P'_2	P'_3	P'_4	Substrate
Ile	Glu	Gly	Arg	=	Val	His	Leu	Thr	*c*IIFXβ-globin
Ile	Glu	Gly	Arg	=	Thr	Ala	Thr	Ser	Human prothrombin[15,16]
Ile	Glu	Gly	Arg	=	Thr	Ser	Glu	Asp	Bovine prothrombin* (ref. 7)
Ile	Asp	Gly	Arg	=	Ile	Val	Glu	Gly	Human prothrombin[16]
Ile	Glu	Gly	Arg	=	Ile	Val	Glu	Gly	Bovine prothrombin* (ref. 7)
Ala	Glu	Gly	Arg	=	Asp	Asp	Leu	Tyr	Human antithrombin III[17]

Proteolytic cleavage by blood coagulation factor X_a occurs between P_1 and P'_1 sites.

* Factor X_a cleaves identically both native prothrombin and its chymotryptic peptides containing these peptide segments.

We first constructed a new expression vector, pLcIIβ, which directs synthesis of a hybrid protein consisting of the 31 amino-terminal residues of λ *c*II protein and the complete β-globin chain under the control of the λ P_L promoter (Fig. 1*b*). A secondary structure near the ribosome binding sequence should be retained even after the β-globin gene has been fused and should provide high translational efficiency. The control plasmid pLcII is identical to pLcIIβ, except that it contains a sequence with multiple restriction sites instead of the β-globin gene insert. When MZ-1 cells (a defective λ lysogenic strain) harbouring pLcIIβ are grown at 30 °C, the *c*I857 temperature-sensitive repressor is active and hence transcription from the P_L promoter is fully repressed[8,9], but at 42 °C the *c*IIβ-globin gene is efficiently transcribed. Figure 2 shows a Coomassie blue-stained polyacrylamide gel of total cellular proteins from cultures with and without heat induction. After heat induction, a new intense band appeared for MZ-1 cells harbouring pLcIIβ. The band co-migrated with soybean trypsin inhibitor consisting of 181 amino acid residues, the same number as in the *c*IIβ-globin hybrid. Western blot experiments[10] showed that the new band, as well as natural human β-globin, reacted with ^{125}I-labelled goat anti-rabbit β-globin IgG (data not shown), indicating that the new band is the *c*IIβ-globin hybrid.

In a second stage, we inserted a short oligonucleotide between the λ *c*II and β-globin genes of pLcIIβ to form pLcIIFXβ, so that the first codon (Val) of the β-globin gene was preceded by a sequence encoding the tetrapeptide: Ile-Glu-Gly-Arg (Fig. 1*b*). This is the sequence that immediately precedes the two factor X_a cleavage sites in prothrombin[7]. The hybrid protein, designated *c*IIFXβ-globin, was purified from MZ-1 cells harbouring pLcIIFXβ and digested with blood coagulation factor X_a which had been activated with Russell's viper venom[11]. Digestion was complete within 2 h at a molar ratio of enzyme to substrate of ~1:100. No further cleavage occurred, except for a slow proteolysis by *E. coli* enzymes which occurred even in the absence of factor X_a. The new band that appeared after factor X_a digestion co-migrated with the slower band of haemoglobin corresponding to the β-chain (Fig. 3). In contrast, the *c*IIβ-globin protein which lacks the tetrapeptide insert remained uncleaved by factor X_a. Reverse-phase HPLC of tryptic peptides confirmed that the product of factor X_a digestion of *c*IIFXβ-globin is identical to natural β-globin (data not shown). Cleavage of the *c*IIFXβ-globin protein by factor X_a occurred exclusively at the peptide bond following Ile-Glu-Gly-Arg, even though the hybrid protein contained 6 other arginyl and 14 lysyl residues. In the same conditions trypsin cleaves β-globin at every peptide bond following arginine and lysine, thus the absence of such cleavage by coagulation factor X_a cannot be due to inaccessibility of the substrate. Moreover, the enzyme

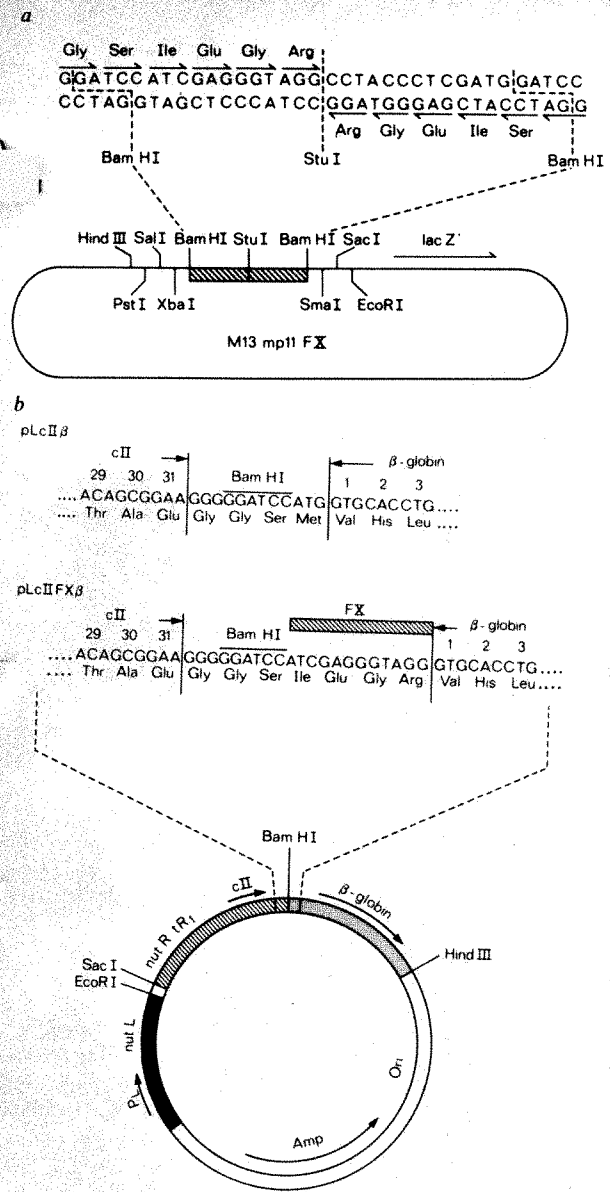


Fig. 1 Construction of M13mp11 FX and pLcIIFXβ. *a*, M13mp11 FX contains a sequence encoding the recognition site for factor X_a. This M13 derivative can be used to join any coding sequence to the factor X_a recognition sequence. *b*, pLcIIFXβ and pLcIIβ direct efficient production of a hybrid protein consisting of the 31 amino-terminal residues of the λ *c*II protein and the complete human β-globin, with and without the factor X_a cleavage site, respectively.

Methods: All DNA manipulations were essentially as described elsewhere[18]. A temperature-sensitive lysogenic strain MZ-1 (*gal*K_{am}Δ*8att*LΔ*Bam*N_7N_{53}*c*I857ΔH1, *his*$^-$, *ilv*$^-$, *bio*$^-$, N^+, given by Dr K. McKenney) was used as a host strain for plasmids containing the λ P_L promoter and transformation was done as described by Remaut *et al.*[8]. T4 DNA ligase was prepared from strain NM989 (refs 19, 20). Restriction enzymes were from New England BioLabs. *a*, M13mp11 FX: Two oligonucleotides, dTACCCTCGATGGATC and dCATCGAGGGTAGGCC, were synthesized by a phosphotriester method on a controlled pore glass support[21] and purified by HPLC[22]. The two oligonucleotides were allowed to anneal after phosphorylation with T4 polynucleotide kinase (P-L Biochemicals) and r[γ-^{32}P]ATP (3,000 Ci mmol^{-1}; Amersham) and ligated to form concatamers. The DNA was then digested with *Bam*HI and cloned into the dephosphorylated *Bam*HI site of M13mp11 (ref. 23) to yield M13mp11 FX which forms blue plaques in the presence of isopropyl-β-D-thiogalactopyranoside and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (Sigma). *b*, Construction of pLcIIβ and pLcIIFXβ: The *Eco*RI–*Hin*dIII fragment containing the multi-restriction sites was cut out from M13mp10 (ref. 23) and ligated to *Eco*RI–*Hin*dIII-cut pLc245 (ref. 8) to form pLmp10. The 319-base pair (bp) *Alu*I fragment containing the *nutR*, t_{R1} sites[3] and a part of the *c*II gene was cut out from pKG1805 (ref. 24) and cloned into the *Sma*I site of M13mp10 in the same orientation with respect to the β-galactosidase α-peptide gene. The *Eco*RI–*Hin*dIII fragment containing the λ DNA sequence was then cut out and cloned into the *Eco*RI–*Hin*dIII site of pLmp10 to yield pLcII. A complete human β-globin cDNA sequence was reconstructed by joining restriction fragments prepared from an incomplete cDNA clone (pJW102)[25] and a genomic DNA clone[26], and this was cloned into the *Sma*I–*Hin*dIII site in M13mp9. The M13mp9 β cDNA thus obtained was opened at the *Nco*I site, which is located at the initiation codon, and treated with Klenow DNA polymerase (Boehringer Mannheim) in the presence of 100 μM 4dNTP to obtain flush ends. The β-globin cDNA sequence was then cut out with *Hin*dIII and inserted into the *Bam*HI (filled-in)–*Hin*dIII site of pLcII so that the β-globin gene was fused to the λ *c*II gene in-phase via a small linker DNA derived from M13mp10. To construct pLcIIFXβ, M13mp9 β cDNA was opened with *Nco*I and 40 μg of DNA were treated with 200 U of mung bean nuclease (P-L Biochemicals) in 30 mM Na-acetate *p*H 4.6, 50 mM NaCl, 1 mM $ZnCl_2$, 5% glycerol at 0 °C for 10 min to remove the 5′ protruding end. The β-globin cDNA sequence was cut out with *Hin*dIII and cloned into the *Stu*I–*Hin*dIII-cut M13mp11 FX. The DNA sequence was determined by the dideoxy chain termination method[27] to ensure that the first valine codon of the β-globin gene was preceded by the DNA sequence coding for Ile-Glu-Gly-Arg. Then, the *Bam*HI fragment containing a part of the β-globin sequence was cut out and cloned into *Bam*HI-digested pLcIIβ to form pLcIIFXβ.

does not require the three-dimensional structure of the substrate to be intact, as it cleaves the recognition sequences in both native prothrombin and in two chymotryptic prothrombin peptides[7]; this shows that it is strictly sequence-specific.

All the polypeptides cleaved by factor X_a contain arginine and glycine at the P_1 and P_2 sites, respectively, and the P_3 site is occupied by a negatively charged residue—glutamic acid or aspartic acid (Table 1). As the peptide bond following Leu-Gly-Arg in *c*IIFXβ-globin is not cleaved by factor X_a, a Glu-Gly-Arg or an Asp-Gly-Arg sequence seems to be a minimal requirement for cleavage. The requirement for the P_4 site is not yet clear, but the present limited data suggest that it must be occupied by a hydrophobic residue. Table 1, together with the data for other trypsin-like serine proteases, shows that the P'_1 site can be occupied by a variety of amino acid residues, including a large aliphatic one, a positively charged one or a negatively charged one. On the other hand, trypsin-like proteases do not cleave peptide bonds preceding proline. Therefore, factor X_a will cleave any peptide bond preceded by Ile-Glu-Gly-Arg or Ile-Asp-Gly-Arg, unless proline occupies the P'_1 site, and may be regarded as a 'restriction endopeptidase'. Several short eukaryotic polypeptides have been liberated from hybrid proteins by trypsin or cyanogen bromide, but such methods[6,12,13] cleave proteins like β-globin at several arginines and lysines or at several methionines.

In our expression/cleavage system the bacterial leader sequence provides a high translational efficiency while the factor X_a cleavage eliminates the extra amino-terminal methionine encoded by the initiation codon which is present in most eukaryotic proteins expressed in *E. coli* by conventional methods[6]. The pro-enzyme, factor X, is readily prepared from bovine blood plasma with a yield of 5 mg l^{-1}. Factor X is isolated biochemically pure and free from contaminating proteases after adsorption from plasma on insoluble barium salts, followed by one chromatographic step[14]. For the construction of other hybrid protein expression vectors, any blunt-ended coding sequence can be cloned into the *Stu*I site of M13mp11 FX which contains a sequence encoding the factor X_a recognition site (Fig. 1*a*). The gene, together with the factor X_a recognition sequence, can then be cut out from the M13 clone with *Bam*HI and cloned into the *Bam*HI site of pLcII, or indeed any hybrid protein expression system, so that the cleavable hybrid protein can be produced. This method is now being applied, in our laboratory, to the production in *E. coli* of several eukaryotic proteins.

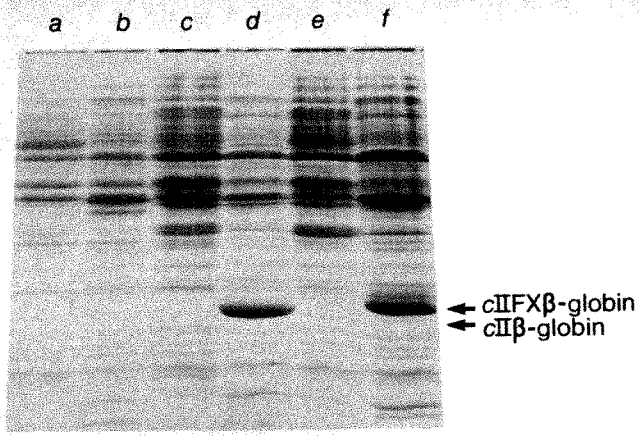


Fig. 2 Expression of *c*IIβ-globin hybrid protein in *E. coli*. Total cellular proteins from MZ-1 carrying pLcII, pLcIIβ and pLcIIFXβ, with and without 2 h of heat induction, were analysed on an 18% polyacrylamide-SDS gel and visualized with Coomassie blue. *a*, pLcII at 30 °C; *b*, pLcII at 42 °C; *c*, pLcIIβ at 30 °C; *d*, pLcIIβ at 42 °C; *e*, pLcIIFXβ at 30 °C; *f*, pLcIIFXβ at 42 °C. **Methods:** MZ-1 cells harbouring the expression plasmids pLcII, pLcIIβ and pLcIIFXβ were grown to $A_{600} = 0.7$ in 2 × TY medium (16 g Tryptone, 10 g yeast extract, 5 g l^{-1} NaCl) at 30 °C and for each of the cultures, one half was mixed with an equal volume of 2 × TY preheated to 65 °C and grown at 42 °C. The other half was grown further at 30 °C as a control. The total cellular protein was extracted with an equal volume of phenol and spun down after precipitation with 5 vol. ethanol[8]. The pellet was dissolved in SDS sample buffer and analysed on an 18% polyacrylamide-SDS gel[28].

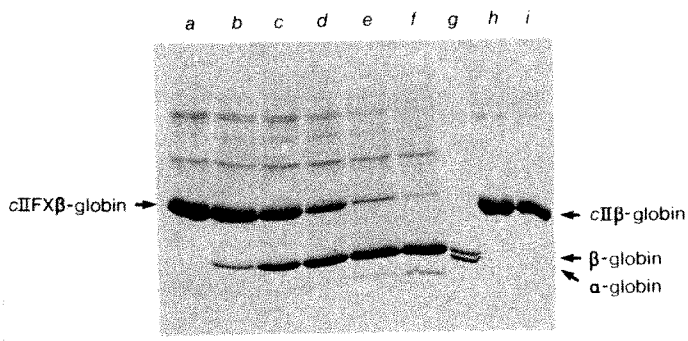


Fig. 3 Digestion of *c*IIFXβ-globin protein with bovine blood coagulation factor X_a. The *c*IIFXβ-globin hybrid protein was digested with factor X_a at an enzyme to substrate molar ratio of 1 : 100 at 25 °C and analysed on an 18% polyacrylamide-SDS gel[28]. *a*, Undigested *c*IIFXβ-globin; *b*, 5 min; *c*, 15 min; *d*, 30 min; *e*, 60 min; *f*, 120 min after addition of factor X_a; *g*, human adult haemoglobin comprising the α (faster band) and β (slower band) chains. The *c*IIβ-globin hybrid protein which lacks the cleavage site for factor X_a was treated with factor X_a in the same conditions: *h*, after 120 min of treatment; *i*, untreated *c*IIβ-globin. **Methods:** MZ-1 cells harbouring pLcIIFXβ were grown in 500 ml of 2 × TY medium. At $A_{600} = 0.7$, the culture was mixed with 500 ml of 2 × TY preheated to 65 °C, then grown at 42 °C. After 2 h, cells were collected, and the high salt precipitate was prepared[29]. The pellet was dissolved in 30 ml of 10 mM Na-phosphate *p*H 6.4, 1% SDS (BDH), 1% β-mercaptoethanol and incubated in a boiling water bath for 5 min. The sample was dialysed against 10 mM Na-phosphate *p*H 6.0, 1 mM dithiothreitol, 0.1% SDS and purified on a hydroxylapatite (Bio-Rad, DNA grade) column[30]. The *c*IIFXβ-globin hybrid protein was concentrated by ultrafiltration (Amicon, PM-10 membrane) then precipitated with 6 vol. acetone/0.1 M HCl to remove SDS. The precipitate was air-dried, dissolved in a minimal amount of 8 M urea, 50 mM Tris-HCl *p*H 8.0 and dialysed against 100 mM NaCl, 50 mM Tris-HCl *p*H 8.0 and 1 mM $CaCl_2$. Bovine blood coagulation factor X (given by Dr M. P. Esnouf) was activated to factor X_a with Russell's viper venom (Sigma)[11].

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Erratum

Expression and amplification of the N-*myc* gene in primary retinoblastoma

W.-H. Lee, A. L. Murphree & W. F. Benedict
Nature **309**, 458–460 (1984).

THE first paragraph on page 460 was printed incorrectly. It should read: 'amplification in neuroblastoma and N-*myc* expression in the retinoblastoma strongly suggests that the N-*myc* gene may belong to the proto-oncogene family. The fact that the N-*myc* gene is amplified and expressed in both retinoblastoma and neuroblastoma is of obvious interest. This is especially so because both tumours originate from neural crest ectodermal cells, although retinoblastoma may develop from a more primitive cell than neuroblastoma[22]. However, we have no evidence to exclude the possibility that genes other than N-*myc* are involved in these two types of tumour formation.'

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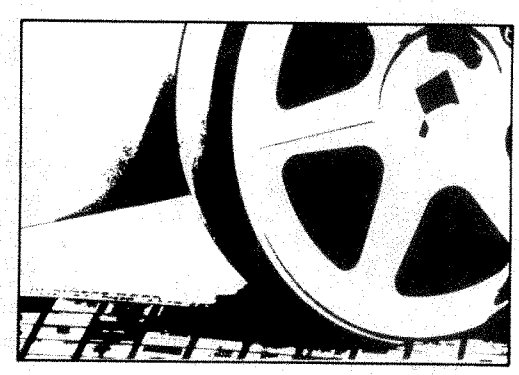
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Science for sale

Dorothy Nelkin

The New Politics of Science. By David Dickson.
Pantheon, New York: 1984. Pp.374. $22.95.

THAT knowledge is power has been well understood and much discussed in many analyses of the politics of science. In *The New Politics of Science*, David Dickson describes the new dimensions of this truism in the 1980s, arguing that science today is a commodity, a policy instrument, a source of legitimation, a basis of imperialism in foreign policy, and a form of property that is being manipulated according to the needs of the military, the marketplace and the private sector. It is a provocative book. Dickson, the European correspondent for the journal *Science*, first characterizes a set of systematic and interrelated changes in American policies concerning science and technology. He then turns to their implications for the nature of scientific research and structure of universities and, more important, to their influence on the relationship between the citizen and the state.

Dickson argues that science is increasingly used to reinforce the concentration of political power in the hands of those who dominate the economy. Tracing the development of patterns of control over science during different historical periods, he concludes that recent changes, reflecting an "almost religious belief in the power of science-based technology", are dramatic and potentially pernicious. Indeed, he has written this book with the openly political purpose of revealing the problematic political and social consequences of recent changes in science and technology policy in the United States. Because of his concentration upon the influence of capitalism, some may interpret the book as merely a diatribe from the left. It is not. Rather, Dickson is pointing to a set of pressures which, taken together, indicate sweeping structural changes in the politics of science.

Dickson's foremost worry is that decisions about both the allocation of scientific resources and the application of scientific results are increasingly concentrated in the hands of corporate banking and military leaders who have gained the willing cooperation of scientific, governmental and academic institutions. He describes how the demands of the private sector influence government research policy and leave social objectives (such as protection of the environment) as issues secondary to the profitability of private corporations. He follows the changing character of budgetary decisions as science is increasingly viewed as "knowledge capital" expected to generate an appropriate return. And he suggests that unfettered technological development is endorsed in order "to get the economy back on its feet", with little attention being paid to its social consequences.

Dickson documents three striking manifestations of the changing politics of science. First are the new relationships between industry and academia that have developed with the renewed commercial interest in basic research. Knowledge, argues Dickson, is a "commodity", a form of "real estate". The result is that the public resources that universities represent are increasingly placed at the service of private capital.

Second, Dickson turns to the renewed links between the American scientific community and the military establishment, as reflected both in changing patterns of military funding of academic research and in growing security controls. Tracing the increased influence of the military in academia since the 1970s, he calls attention to the ironies of scientists accepting — and indeed soliciting — military support while worrying about controls on open communication as the government seeks to limit the transfer of technological information in the interests of national security.

Third, through numerous examples, he points to the growing foreign policy implications of science and technology, given their importance in international economics. Influenced by multi-national corporations, Third World politics are especially vulnerable to policies that are determined by profit rather than need. So too are international agreements such as the Law of the Sea.

Dickson finds it notable that today's politics of science developed after a decade of challenge and introspection during which many people, through the environmental and consumer movements, questioned the definition of "progress" and insisted that science and technology must serve the needs of society rather than determine those needs. That period of protest revealed two conflicting approaches to the politics of science based on "democratic" and "technocratic" strategies. The technocratic approach called for "rational" solutions to the problems of technology through a consensus of experts; the democratic strategy looked towards redistribution of political power through greater public involvement in technological decisions.

For a brief period, demands for increased democratic control over technology appeared to be influencing the politics of science and technology as public agencies groped for assessment procedures that would involve citizens in general. The new politics of science represents a powerful counter-attack against such reforms. Today, Dickson argues, technocratic strategies prevail as technical and economic decision-making methodologies are used to cloak political judgements in the garb of scientific neutrality; and scientific arguments are used to dismiss challenges from concerned citizens. In effect, science has become a policy instrument reinforcing the ascendance of technocratic over democratic values and reducing political choice.

What are the consequences of the new politics? Dickson sees a declining interest in the environmental, social and ethical implications of research. And as criteria of efficiency replace the emphasis on equity, he sees a re-direction of research away from social priorities. He also discerns changes within the community of science as commercial and military interests lead to secrecy and to tensions over traditional patterns of scientific communication. He worries about relationships within universities as certain departments compete to keep their faculty together in the face of well-paid entrepreneurial temptations. But, above all, Dickson worries about the political consequences of the new politics of science — the reduced possibilities for public involvement in political decision-making and the increased deference to technical expertise. Indeed, he sees the future of democracy itself as being at stake.

Dickson's argument is supported by many case-histories and placed in perspective with historical comparisons. While he is convincing, he also raises a number of unanswered questions. How did such dramatic political changes take place so quickly with so little public response? Why are academic scientists, who only a decade ago were widely disenchanted by university–military relationships, so quick to renew those relationships today? Why are campuses and grass-roots movements, until recently mobilized around related issues (military research in universities and the environmental and social effects of technical change), now so apathetic? How have the actors involved in the new politics of science cultivated the public apathy that has allowed uncritical deference to technocratic expertise? Dickson says too little about the media as a vehicle for gaining public consent for the new politics of science, and does little to explain the pressures within science that foster changing attitudes.

Dickson is by no means anti-science or opposed to technological change. Rather, he wants to re-integrate public needs and aspirations into science policy, to shift the priorities of science and technology away from destructive ends (such as defence) and

towards socially constructive goals (such as better health or nutrition). He concludes with some general proposals. He would like to democratize procedures within the scientific community so that priorities and practices would be open for broader public discussion; he would like to change the institutions responsible for allocating research funds to allow greater public input; he wants to develop ways of democratizing technological innovation to ensure that considerations of equity influence production; and he wants to maintain public access to the fruits of publicly funded research.

Dickson ends his book on a bizarre note, by taking encouragement from the effectiveness of the Creationists in challenging scientific ideas. In my view, he misinterprets the significance of Creationism. Far from creating a "political program that includes a vision of an alternative science", this group has manipulated the populist tendencies of American Fundamentalists to enhance the power of a small religious group. Yet this conclusion to the book should not divert the reader from its very positive, indeed Utopian message — that science, one of the greatest cultural and intellectual achievements of the modern age, must be shaped in accordance with democratic values in order to enhance the public good. □

Dorothy Nelkin is a Professor in the Cornell University Program on Science, Technology and Society, and a Visiting Scholar at the Russell Sage Foundation, New York. She is author of Science as Intellectual Property (*Macmillan, 1984*), *and, with Michael S. Brown, of* Workers at Risk (*University of Chicago Press, 1984*).

Metallic electrons

Michael Springford

Magnetic Oscillations in Metals.
By D. Shoenberg.
Cambridge University Press: 1984. Pp.570. £55, $97.50.

OCCASIONALLY a book appears of which it may be said that the author, the content and the timing could not be bettered. *Magnetic Oscillations in Metals* by Professor David Shoenberg is just such a book.

It was in 1932, at the suggestion of Kapitza, that the author, then a new graduate student at the Royal Society Mond Laboratory in Cambridge, was introduced to the mysterious magnetic properties of bismuth. Only a few years previously, in Leiden, de Haas and van Alphen had observed magnetic oscillations in this metal at low temperatures. The oscillations of the magnetization as the magnetic field was varied were thought at first to be peculiar to bismuth, but in time they were understood to be a fundamental property of all metals.

In a historical introduction, Shoenberg charts the course of the development of the de Haas–van Alphen effect, as it came to be called, from a curiosity to arguably the single most important experiment in solid state physics. We read of the insights of Landau on the origin of the effect, of the unexpected success of the nearly free electron model as crucial evidence in the development of pseudopotential theory, and of the subtle and fruitful interplay over the years between experiment and theory. The influence exerted on the course of solid state physics by studies of magnetic oscillations originated in large measure from their versatility and accuracy. Band theoretic methods, for example, could be fine-tuned and became more useful and reliable instruments in other fields. For more than half-a-century, in events from which the author has seldom been far removed, magnetic oscillations have been employed as a spectrometer for the investigation of electrons in metals. It is with these many aspects of the field that the book is principally concerned.

An objective early in the book is to expound the theory of magnetic oscillations, broadly along the lines first set out by Lifshitz and Kosevich in 1955, use being made of the now-familiar geometrical interpretation of electronic properties for the physical insight it provides. The theory is developed for both two- and three-dimensional systems, with the more cumbersome mathematical results being helpfully placed in appendices. Given the historical development of the subject it is proper that the determination of Fermi surfaces (Fermiology), should have a prominent place. Here Shoenberg concentrates more on general principles, following this with a survey of what has been accomplished in a few selected metals. For a more encyclopaedic approach the reader is referred elsewhere.

In later chapters, attention is focused on more contemporary topics where, in characteristic style, the author does not fail to highlight areas where uncertainties remain, such as the effect of dislocations upon magnetic oscillations, nor those, such as the influence of many-body interactions, where even the general principles involved are still very much in question.

Although the study of magnetic oscillations has reached maturity as an established branch of solid state physics, this is remarkably the first book to be devoted to a complete exposition of the subject. Work on magnetic oscillations is still actively pursued and the author believes that this will continue for a long time yet. So too will his book remain the definitive text for research workers in this field. □

Michael Springford is Professor of Experimental Physics at the University of Sussex.

Cultural influence

Paula Brown Glick

Ruth Benedict: Patterns of a Life.
By Judith Modell.
Chatto & Windus/University of Pennsylvania Press: 1984. Pp.355. Hbk £15, $30; pbk $11.95.

RUTH BENEDICT (1887–1948) has occupied an important place in the history of American anthropology for the past 50 years, ever since the publication of *Patterns of Culture* in 1934. Judith Modell, the author of this biography, is herself an anthropologist and quite clearly feels a strong personal identification with her subject. Yet she does not attempt to evaluate Benedict's scholarship or contribution to anthropology.

The biographer is able to cite Ruth Benedict's published and unpublished writings — poems, notebooks, letters and an autobiographical fragment written in 1935 — in her examination of Benedict's childhood and youth. But now, over 30 years after Benedict's death, recollections culled from friends, relatives and students do not fully satisfy inquiry — many questions remain. From the characterization of Stanley Benedict, her husband, and of some close friends, the figures remain shadowy; here the reader must accept the limitations of the biographer's materials. Modell has attempted to discern the pattern of Benedict's life, and has certainly laid out the whole sweep of her person and her interests, as child and adult, in literature and anthropology. The influence of philosophical and religious thought upon Benedict is not fully examined, however; her feminism is muted. And a chapter on her anthropological work was evidently unduly influenced by Margaret Mead's books about Benedict, and seems uncritically repetitive.

Ruth Benedict was a student of Franz Boas of Columbia University, who has been called the "builder and architect of modern anthropology" and who built the Anthropology Department at Columbia University into a centre for anthropology, ethnography and folklore study. Ruth Benedict entered this realm in 1921, in her thirties, there finding a personal role, a purpose for her life and a new subject for writing. She visited American Indian settlements to record texts and cultural data from informants — the usual method of American anthropologists of the time — and was Boas's closest assistant for 20 years, teaching and supervising students

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- *A Feeling for the Organism: The Life and Work of Barbara McClintock* by Evelyn Fox Keller. (W.H. Freeman, $8.95, £7.95.) For review see *Nature* **304**, 377; 1983.
- *Darwin and His Critics* by David L. Hull. (University of Chicago Press, $15, £12.75.) For review see *Nature* **248**, 285; 1974.

although she had no permanent academic position at Columbia.

Boas's women students, especially Margaret Mead and Ruth Benedict, became widely known to American students and scholars in fields far beyond the small group of cultural anthropologists of the time. While Mead married and made many field trips with her husbands, Reo Fortune and Gregory Bateson, and later with students, Benedict remained at Columbia. There she edited the *Journal of American Folklore,* taught and published. Only after she separated from her husband and lived independently in New York did she have a regular post at the university.

Benedict's best-known work, *Patterns of Culture,* was the culmination of her interests in coherence of culture as a design for living. The work has long been recognized as an anthropological landmark. It developed from Boas's concern with ethnographic detail to the integration of a whole "culture". This notion that a culture, the total way of life of a people, could be characterized in a single word, Apollonian or Dionysian, was Benedict's unique insight; Modell feels it is her real achievement. Such a breathtaking whole view could not be achieved as a characterization of very many of the cultures studied by anthropologists, and this difficulty with the concept has been noted by a number of critics. However, the identification of themes and the relations between the individual and cultural values became one of the main lines of anthropological inquiry and had wide influence. At the beginning of the Second World War, Benedict and Mead, with a group of anthropologists and psychologists, carried out studies of the personality and culture of contemporary peoples, especially in European and Asian nations. The best-known of these studies, Benedict's *The Chrysanthemum and the Sword,* became a handbook used by American occupation forces in Japan after the Second World War.

Benedict died in 1948 at the age of 61, Mead and others completing the work on national character. Since the early 1950s, however, the attempt to identify a single cultural theme or pattern has been criticized as oversimplification. This, then, the outstanding contribution of Benedict to anthropology, was not followed up successfully by the next generation of scholars. Benedict's approach to anthropology as one of the humanities declined as cultural and psychological anthropology became more scientific in its methods and results.

The discipline of anthropology may indeed have progressed beyond the goal of identifying a unitary cultural theme. Nonetheless the person and concepts of Ruth Benedict, as presented here by Judith Modell, are of lasting interest and value to students of life and of culture. □

Paula Brown Glick is Professor of Anthropology at the State University of New York, Stony Brook.

States of mind and of science

Peter Wason

From Folk Psychology to Cognitive Science: The Case Against Belief.
By Stephen P. Stich.
MIT Press: 1984. Pp.266. $22.50, £21.40.

THE ordinary reader might expect to learn from this book something about the so-called cognitive revolution which has transformed the science of psychology over the past few decades. After all, the title suggests something of this kind. Like folk medicine, folk psychology carries an unscientific, even a disreputable, connotation; it conjures up an image of untutored individuals who lack access to the sources of enlightenment. On the other hand, cognitive science has an honorific connotation, without the parochial aura of psychology and suggesting a lot of brainy people from some of the new disciplines (artificial intelligence, for example) all toiling together happily on fundamental epistemological problems and talking a language nobody else can understand.

The ordinary reader would not be entirely right. Dr Stich is not concerned with psychological research, but with a philosophical argument about the status of the mind and its cognitive capacities. His main concern is to propose that the concept of belief (and allied mental concepts) has no proper function in a scientific explanation of mental life. We have all got things wrong if we want to be taken seriously as scientists. Neither folk psychology, nor cognitive science, is accurately defined, but the former is assumed to comprise "a loose network of largely tacit principles, platitudes, and paradigms" based on mentalistic terms. The author's argument is that such folksy notions, re-introduced into cognitive psychology after the bleak days of behaviourism, are detrimental to scientific progress and have no place in scientific accounts. This echoes in a strange way Otto Neurath's *International Encyclopedia of Unified Science* (1955), with its aim of purging scientific terms of surplus meaning. It is strange because positivism is hardly considered respectable in advanced philosophical circles and certainly not among the adherents of cognitive science. It is not simply in politics that left and right end up advocating the same thing.

Although the author draws back from giving whole-hearted assent to the claim of a couple of other philosophers that folk psychology is a "degenerating research program", he would like to rule out from cognitive science any "content sentence" which expresses an intentional or propositional attitude. Thus none of the following gain admittance:

S believes that Ouagadougou is the capital of Upper Volta
S hopes that Ouagadougou is the capital of Upper Volta
S fears that Ouagadougou is the capital of Upper Volta
S desires that Ouagadougou is the capital of Upper Volta,

for the folk psychological use of terms such as "believes", "hopes", "fears" and "desires" do not "play a role in the best and most sophisticated theories put forward by contemporary cognitive scientists". The precise nature of these admirable theories remains unspecified, but one might raise the question of how a clinician can make a diagnosis of paranoia without reflecting upon the beliefs of the patient. Further, one might also point out that there are philosophers who have made interesting analyses of emotions in ordinary folk terms.

What are the proposals which the author commends in the place of such crude

The Tables Turned: An Evening Scene

Up! up! my friend, and clear your looks,
Why all this toil and trouble?
Up! up! my friend, and quit your books,
Or surely you'll grow double.

The sun above the mountain's head,
A freshening lustre mellow,
Through all the long green fields has spread,
His first sweet evening yellow.

Books! 'tis a dull and endless strife,
Come, hear the woodland linnet,
How sweet his music; on my life
There's more of wisdom in it.

And hark! how blithe the throstle sings!
And he is no mean preacher;
Come forth into the light of things,
Let Nature be your teacher.

She has a world of ready wealth,
Our minds and hearts to bless —
Spontaneous wisdom breathed by health,
Truth breathed by chearfulness.

One impulse from a vernal wood
May teach you more of man;
Of moral evil and of good,
Than all the sages can.

Sweet is the lore which nature brings;
Our meddling intellect
Misshapes the beauteous forms of things;
— We murder to dissect.

Enough of science and of art;
Close up these barren leaves;
Come forth, and bring with you a heart
That watches and receives.

This poem, by William Wordsworth, is taken from *Poems of Science*, a new anthology published by Penguin. The book is edited by John Heath-Stubbs and Phillips Salman, and among the poets represented are Shakespeare, Milton, Shelley, Walt Whitman, Louis MacNeice and John Updike. Price is £4.95 in paperback.

notions? His favoured option, carefully argued against other candidates, is "The Syntactic Theory of Mind" (STM). It is introduced in these words:

> The basic idea of the STM is that the cognitive states whose interaction is (in part) responsible for behaviour can be systematically mapped to abstract syntactic objects in such a way that causal interactions among cognitive states, as well as causal links with stimuli and behavioural events, can be described in terms of the syntactic properties and relations of the abstract objects to which the cognitive states are mapped. More briefly, the idea is that causal relations among cognitive states mirror formal relations among syntactic objects. If this is right, then it will be natural to view cognitive state tokens as tokens of abstract syntactic objects.

I do not find this altogether intelligible, and not just because I fail to understand how objects can have a syntax. I do not really grasp how a theory such as this (however elaborated) could illuminate mental processes, and particularly the ways in which they go wrong in real life. However, it will be retorted (rightly) that the theory is not *really* a theory at all, but a kind of prolegomenon for a cognitive theorizing. One might suppose, then, that it is meant to be a methodological prerequisite for research, on the analogy with the methodologies for behaviourism which were proposed in the 1940s and 1950s. But there seems to me an interesting difference between them. The philosophers of science who analysed the concepts of behaviourism, as well as the concepts of the physical sciences, based their research on the use of the concepts within the theories. Philosophers of mind tend to ignore previous theory and appeal to their own insights, conjectures and intuitions, paraded forth in a series of pregnant sentences. There is nothing wrong with this, and it is often quite effective in advancing an argument. But the logical precision achieved in this way is also likely to be much more puzzling to the non-specialist than was the more discursive work of some of the great philosophers of the past.

There is a real danger that philosophers will end up talking only to each other and abandon their traditional role of protecting us from conceptual confusion. My doubt about the present book is whether its thesis will be of interest to experimentalists. It is a doubt rather than a verdict. Although Stich's arguments are nearly always compellingly precise, I am unsure whether they are consequential, or merely dazzling moves in an intellectual game. In the nature of things, they will not be readily settled because assertions about states of mind are not to be verified like assertions about nerve impulses. So while this book may be of interest to philosophers and to cognitive scientists, it remains uncertain whether it will help the rest of us in trying to find out more about mental processes. □

Peter Wason is Reader in Psycholinguistics at University College London. With P.N. Johnson-Laird he is co-author of Psychology of Reasoning: Structure and Content *(Batsford, 1972).*

Talk of research

Augustine Brannigan

Opening Pandora's Box: A Sociological Analysis of Scientists' Discourse.
By G. Nigel Gilbert and Michael Mulkay.
Cambridge University Press: 1984. Pp.202. Hbk £22.50, $37.50; pbk £7.95, $12.95.

OVER the past decade, a close descriptive approach to scientific behaviour has revived interest in the cognitive and social dimensions of contemporary scientific knowledge. Following in this direction, the Pandora investigation focused on a recent controversy in biochemistry. The study is based on intensive interviews with 34 bioenergists in the United States and Britain, as well as on a sample of their correspondence and a reading of the literature in the field between 1960 and 1980. This period witnessed the rise of competing theories to explain how the crucial ATP molecule was able to move and temporarily store energy in living cells. In 1978 Peter Mitchell was awarded a Nobel Prize for chemistry for his work on the role of oxidative phosphorylation in ATP formation.

However, research by the authors indicated that members of the "ox-phos" community, as it is called, were far from in agreement about what exactly had been discovered in chemiosmotic research, about the empirical validity of the various bits of evidence, and about the respective parts played by the leading contributors. In their travel between the various biochemical laboratories, Gilbert and Mulkay report they were struck by the fact that although they realized that they were being given radically different accounts by participants of what "really" had happened, each account seemed, on its own, plausible and convincing.

Rather than trying to find out who was "really" correct and who was in error, the sociologists, not being in the business of second-guessing biochemical questions, turned their attention to what they describe as "discourse analysis". This is what opens Pandora's Box. Discourse analysis requires that the sociological observer remains neutral as to who is "actually" correct, the object being to determine *how* the respective participants produce and maintain their own sometimes contradictory versions of reality in the face of what would seem to be a set of invariant natural facts.

The Pandora investigation was not undertaken as a critique of science, for Gilbert and Mulkay go well beyond observations of how scientists selectively expose themselves to natural facts and selectively perceive and retain them. The heart of their inquiry is the observation that scientists employ two repertoires or codes of discourse for talking about how nature is discovered. The familiar empiricist frame of mind explains progress as following naturally from adherence to careful observation, and the testing of rival hypotheses in controlled experiments. In addition to this, scientists also employ what is called a "contingent" repertoire in which progress is traced to unique, subjective or situationally specific factors. Gilbert and Mulkay illustrate the use of these two conflicting repertoires in a fascinating contrast between the informal factors that influenced the investigation and interpretation of "ox-phos" experimental data reported to them in interviews, and the formal rationality provided in the earlier published articles of the same researchers. Rather than siding with the informal "inside" story as the true one, they instead stress that the accounts given of actions vary with the needs of the circumstance in which they are produced. Different accounts draw from the original experience in different ways depending on the formality or informality of the situation.

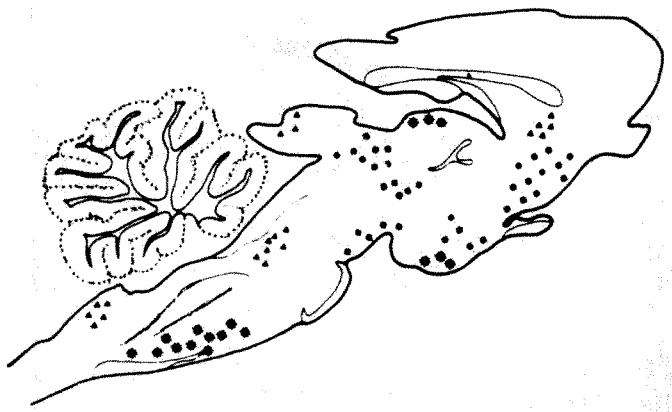
Having made a convincing case for the existence of two separate repertoires for reporting the progress of chemiosmo theory, the authors then go on to suggest that these repertoires are employed to explain the differential acceptance of scientific beliefs across the community. While one would expect that consensus ought to follow closely the reading of the facts, this is not the case. The evidence shows that scientists typically ground their own beliefs in empiricist discourse, as following from the experimental results, while explaining the position of opponents in the contingent repertoire: opponents wrongly interpret the facts because of subjective bias, experimental incompetence, theoretical ignorance — and even national origins! This switching between the two repertoires virtually guarantees that opponents will talk past one another. According to the authors, the device which saves the community from solipsism is the belief that in the end "the truth will out" — though how, when and in whose favour, no one can determine beforehand.

Other highlights of this work include an outstanding analysis of the tension between the virtual versus the fictional elements in scientific drawings of the chemiosmotic mechanisms. One informant dubs them "working conceptual hallucinations". There are also discussions of consensus and the significance of scientific humour. Each of these topics is explored usefully in the context of the two-repertoire dichotomy. While based on a single scientific speciality, the lessons of this investigation probably have wide currency in all areas of science, especially where controversy mobilizes the two incommensurable structures of discourse identified here. For that reason the book will be read with profit by practising scientists as well as by sociologists of scientific knowledge. □

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Frontiers in Neuropsychiatric Research

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Based upon the proceedings of a CINP satellite symposium held in Corfu in June 1982, this book brings together contributions from the many fields involved in psychiatric research

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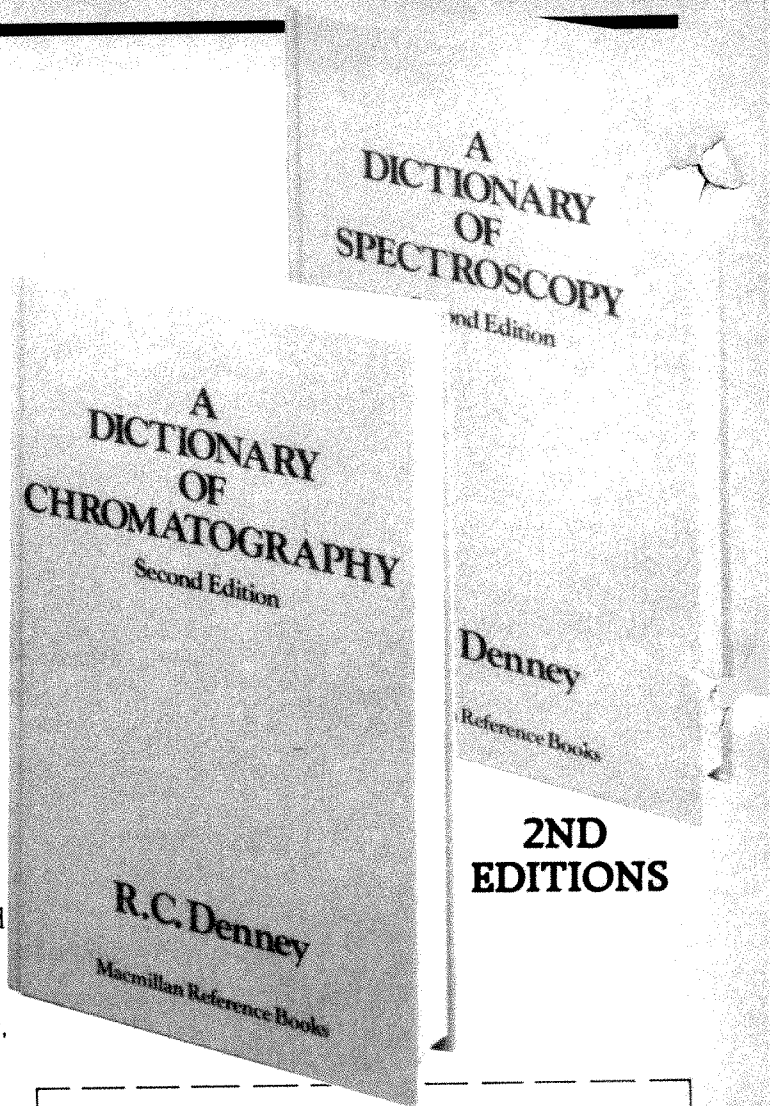
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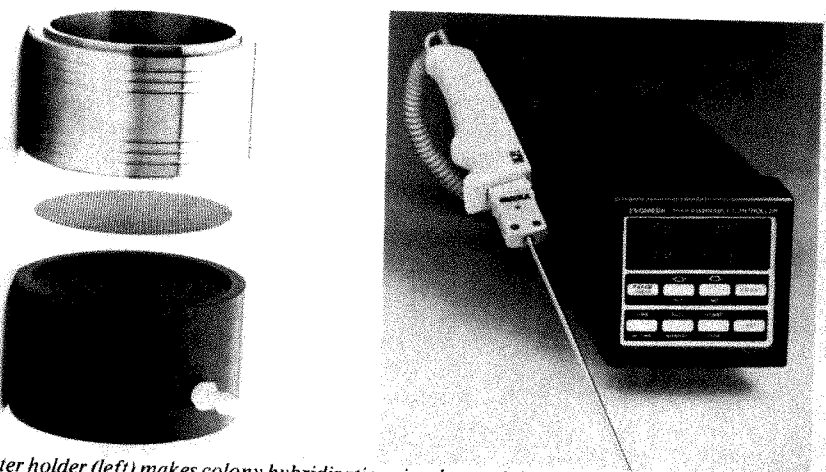
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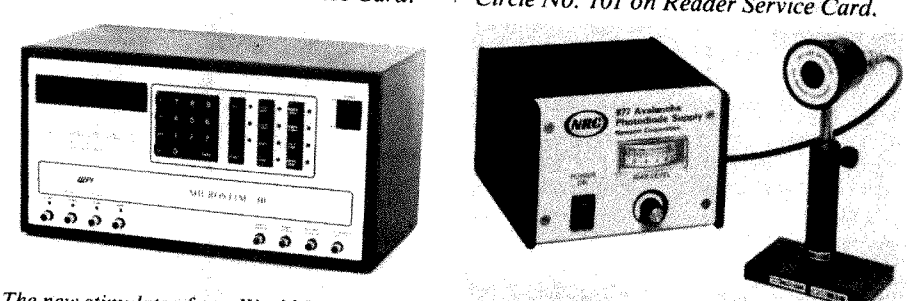
A new filter holder (left) makes colony hybridization simpler, and the Omega CN2010 does the same for temperature control.

● The Newport Model 875 high-speed PIN photodetector has broad spectral sensitivity from 400 to 1,060 nm and sub-nanosecond risetime. The detection area is large and is easy to align in a beam. An optional optical fibre input is available. The Model 877 ultra-high speed APD photodetector has rise and fall times of less than 200 picoseconds. It is useful for characterizing laser diodes as it is designed to detect and resolve ultra-short pulses of visible light. Both feature rechargeable battery power supplies.
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● Marubishi bench-top cell cultivation fermentors for animal and mammalian cells are now available in the United States from Bioengineering Associates Inc. These fermentors are suitable for a wide range of cell types and methods of cell cultivation, including cell suspension, microcarriers, hybridoma and plant cells. Agitation is produced gently by a marine-type propellor driven by magnetic coupling to eliminate any penetration of the fermentation chamber. Automatic temperature control within 0.5°C is achieved by a proportional controller balancing the output of an electric heating unit with the flow of a cool water circulating system.
Circle No. 105 on Reader Service Card.

● New from Pharmacia P-L Biochemicals, "Gene cartridges" are a group of promoters, antibiotic resistance genes and multi-site linkers used to custom-tailor cloning vectors to the customer's specifications. The range includes cloning vectors, oligonucleotides, molecular biology enzymes and restriction nucleases, all listed in the Pharmacia PL "Product Reference Guide".
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● The new Omega CN-2010 programmable temperature controller features a "ramp and soak" function, which controls the temperature and its rate of change over a predetermined time span when more than one temperature is required to complete a process. Up to eight ramp and soak intervals can be programmed, each with a time duration of up to 254 repeat cycles. The CN-2010 has a multisetpoint function that allows presetting of up to nine independent setpoints. The controller accepts any standard thermocouple, RTD sensor, d.c. voltages up to 200 V or d.c. currents up to 200 mA. It will also interface with a host computer via an RS-232C, RS-422 or 20 mA current loop, allowing the user to access all input parameters and output values and assume direct control of outputs.
Circle No. 101 on Reader Service Card.



The new stimulator from World Precision Instruments (left) and Newports' photodetector.

● Polypropylene disposable bags for autoclaving and sterilizing contaminated products are available from Sarstedt Inc. The bags are printed with prominent biohazard labels and come in three sizes. They can be autoclaved at up to 148°C.
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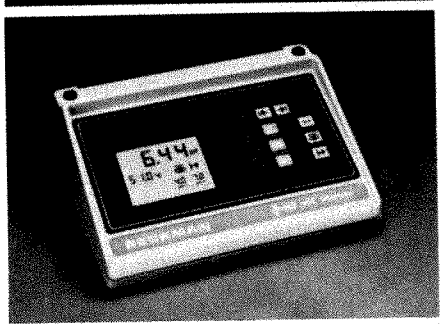
● Tekspensor adjustable volume dispensers from Tekmar use micrometer adjustment for precise repeatability to dispense volumes from 0.4 to 100 ml with no mechanical slippage. Accuracy and reproducibility are better than ± 0.1%. The dispensers are made of chemically inert materials and the calibrated piston, suction tube and ejection tube are PTFE coated to minimize freezing problems associated with all-glass dispensers.
Circle No. 109 on Reader Service Card.

● The Bio-Rad Model 1305A variable wavelength UV monitor is a detector designed for chromatographic applications which require high sensitivity with low noise and drift. The monitor provides digital readout of absorbance, transmittance, or photo detector output independent of the recorder. The standard deuterium lamps allow a 190 to 350 nm wavelength range. The monitor may be upgraded by adding the optional tungsten lamp which extends the range from 350 to 600 nm. Sensitivity is selectable in eleven steps from 0.005 to 5.12 ABS/full scale. A time constant switch permits selection of a fast or slow response time. The monitor features separate electronic and optical modules to conserve bench space, and convenient interchangeable cassette-type flow cells for analytical, preparative, microbore, ultra-microbore and classical open column chromatography. The Model 1306 UV monitor is a simpler device for applications which require detection in the 190 to 350 nm wavelength only. The optical and the electronic components are located in the same unit, which makes this detector more compact than the Model 1305A.
Circle No. 110 on Reader Service Card.

● Chromatographic data can be acquired, analysed and reported using the new Adalab chromatograpy data system, Chromatochart and an Apple II computer. Data can be acquired from capillary and packed column gaschromatographs, HPLCs, gel scanners, TLC densitometers and amino acid analysers. It integrates and provides LC gradient control on four channels simultaneously, storing methods, raw data and reports on floppy disk.
Circle No. 111 on Reader Service Card.

● The new shaking waterbath from Haake Buchler Instruments Inc. is designed for gently agitating laboratory samples while holding them at a controlled temperature, as well as for specialized uses such as thawing frozen plasma or whole blood. The entire volume of the stainless steel bath is usable, since the heater and sensor are mounted below the bath. A large area heater reduces hot spots and provides even heating throughout the bath.
Circle No. 112 on Reader Service Card.





The Sarstedt disposable bag for waste (top) and Beckman's new pH meter.

● New from the National Appliance Company is a thermostatically-controlled waterbath capable of maintaining temperatures of 100°C when operated with or without a cover. Thermostatic controls allow use at lower temperatures, as well. A limiting thermostat protects the heater element from low-water conditions.
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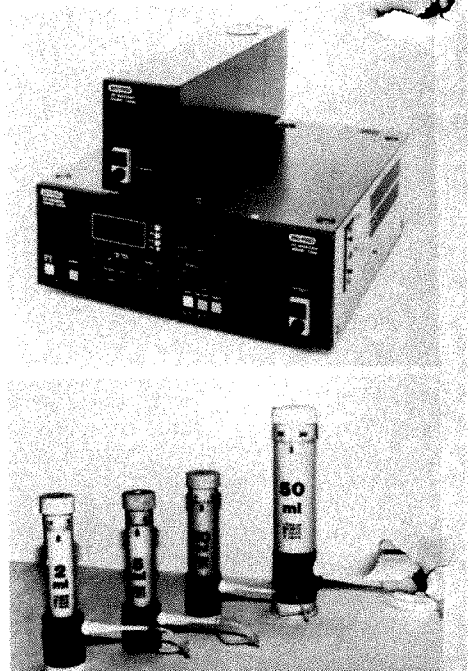
● Falcon Labware has introduced new 17 × 100mm round-bottom tubes for use in the collection processing, storage and centrifugation of biological specimens. The tubes have graduations for easy volumetric reference and are available fabricated from either polypropylene or polystyrene. The tubes are delivered sterile with a patented dual-position cap. The first position closes the tube but permits aerobic culturing of microbiological specimens. The second position fully seals the tube allowing it to be used for specimen storage and centrifugation.
Circle No. 114 on Reader Service Card.

● Standardized nucleic acid and immunological reagents for oncogene research are now obtainable from the North-London based company, Stratech Scientific Ltd. These highly purified, sequence specific DNA fragments have been designed for use in nick translation reactions and for the detection of oncogenic sequences by nucleic acid hybridization. Also available are antibodies for the detection of oncogene protein products and related antigens.
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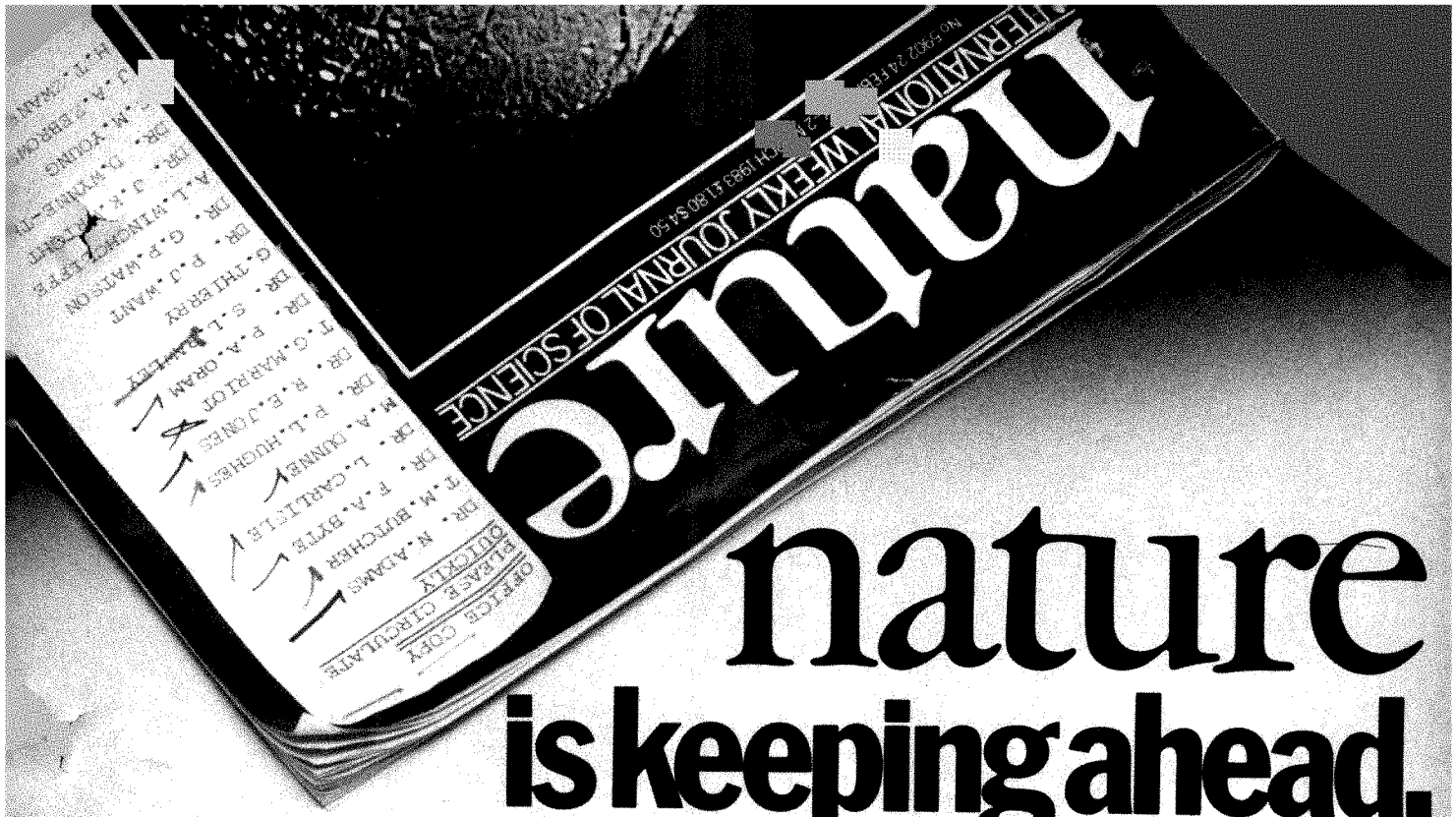
● Intended for routine and complex HPLC application, Gilson's new variable wavelength UV detector permits easy user programming of all instrument parameters. Operating from 190 to 380 nm, the Model 116 features a sensitivity range of 0.001-2.0 AUFS adjustable in increments of 0.0001 AUFS. The user can monitor at two different sensitivities simultaneously. Other features include a build-in keypad and computer interface.
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● Human leukocyte interferon (α-IFN) intended specifically for AIDS research can now be obtained from Schwarz-Mann. It is prepared from normal human buffy coat cells induced by Sendai virus, purified by a modified Cantell method. The preparation is free of Sendai virus when assayed by haemagglutinin after serial passage through chicken eggs, and is free of hepatitis B surface antigen. Anti-human interferon α, purified human leukocyte interferon (α-IFN) and anti-human interferon β are also available.
Circle No. 117 on Reader Service Card.

● The Beckman PhI 40 bench-top *p*H meter is a simple-to-use instrument for accurate *p*H measurement in quality control, environmental and research laboratories. A custom-designed integrated chip gives the meter the sophistication of more costly models while making it easy to use. Most measurement steps are automated for nearly unattended measurement and simple operation. It has error and out-of-temperature-range warnings; always displays temperature; and does temperature computations with or without an automatic temperature compensation (ACT) probe. It features a digital-to-analog converter for simplified recorder setup.
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The Bio-Rad 1305A (top) and Tekspensors from Tekmar.



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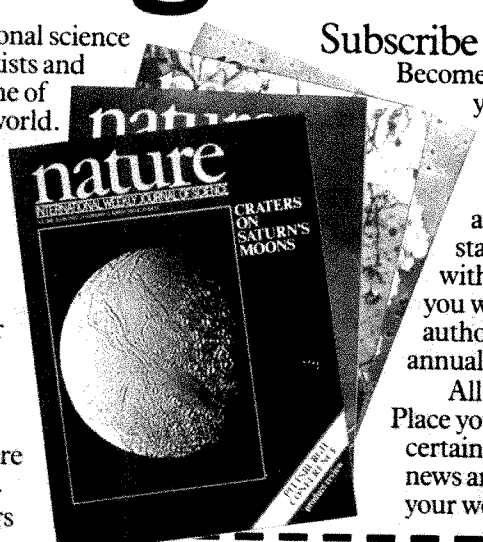
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UNIVERSITY OF ABERDEEN

DEPARTMENT OF FORESTRY

RESEARCH ASSISTANT (Ref 2)

Required for a 15-month NERC-funded project on tissues and protoplast culture of alder and birch as part of a wider study of the application of biotechnology to the improvement of forest trees. Candidates should have a good honours degree in Botany, Biochemistry or allied discipline with experience of in vitro culture methods.

Salary on the Range 1B scale for Research and Analogous Staff (£6,310 — £8,530) with appropriate placing (scale under review). The appointment is tenable until December 1985.

Further particulars and application forms from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 27 July 1984. Please quote Ref No 2 on application. (3067)A

THE UNIVERSITY OF MANCHESTER

DEPARTMENT OF ASTRONOMY

MANAGER OF THE STARLINK NODE

Applications invited for the above post to manage the Manchester Node of the Starlink network. Duties will involve the smooth running of the node, to assist users with problems and to contribute in the development of software for the Starlink project.

Applicants should have a degree in a numerate subject, at least three years of relevant computing experience. An interest or background in Astronomy would be an advantage. Post tenable initially for two years from October 1st, 1984 or as soon as possible thereafter.

Salary range pa: £7,190 — £11,615 (Superannuation). Particulars and application forms (returnable by July 20th) from the Registrar, The University, Manchester M13 9PL. Quote ref 151/84/N. (3058)A

HARVARD UNIVERSITY PEABODY MUSEUM

DEPARTMENT OF ANTHROPOLOGY

seek applicants for a newly-endowed Landon T Clay

PROFESSORSHIP OF SCIENTIFIC ARCHAEOLOGY

Applicants should have well established archaeological interests and be scientists with active research programs. In addition to pursuing his/her own research, the appointee will be encouraged to develop and direct a laboratory engaging in varied applications of the natural sciences to archaeological problems.

Women and minority group candidates are especially encouraged to apply.

Send detailed vita to Chairman, Department of Anthropology, Harvard University, Cambridge, MA 02138, by November 30, 1984. (NW644)A

UNIVERSITY OF LEICESTER SCHOOL OF MEDICINE

TEMPORARY LECTURESHIP IN ANATOMY

Applications are invited for a Temporary Lectureship in the Department of Anatomy tenable for one year from 1 November 1984. The successful candidate will be required to have a knowledge of topographical anatomy. The post is suitable for a scientist wishing to embark upon a career in anatomy or for a medical qualified graduate intending to the Primary FRCS.

Initial salary will depend upon qualifications and experience on the scale £7,190 to £14,125 (under review).

Further particulars from the Registrar, University of Leicester, University Road, Leicester LE1 7RH, to whom applications should be sent on the form provided by 31 July 1984. (3037)A

Senior Research Scientist

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Smith Kline and French is a leading international pharmaceutical Company with a substantial commitment to research and development in the UK. Our laboratories at Welwyn, Herts are well equipped and amongst the most advanced in our field.

As part of our thrust towards the discovery of new medicines we are looking for a man or woman to join our team and contribute to the further development and direction of our cardiovascular research programmes.

The person we need should have experience in aspects of intracellular control of cardiac and/or vascular function and who can combine a range of scientific understanding to the solution of problems in this challenging area of research.

Applicants should possess a suitable qualification in life sciences combined with substantial research experience obtained in either academia or industry. There is plenty of scope for personal development.

Please ring 07073 25111 extension 3540 for further information or an application form or send a detailed cv (ref no N/973/TF) to Dr T E Forster.

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(3095)A

MACQUARIE UNIVERSITY SYDNEY AUSTRALIA

LECTURER IN BIOLOGY

LECTURER IN PHYSICS

Equality of Employment Opportunity is University Policy.

Applications are invited for appointment to the following lectureships tenable not before 1 February 1985 for a fixed term of five years.

Salary (under review) $A24,353 to $A31,994 p.a.

Further information about the University and advice regarding the method of application should be obtained from the Secretary-General, Assocation of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, England, or from the Registrar, Macquarie University, North Ryde, New South Wales 2113, Australia.

Applications close 3 August 1984.

LECTURER IN BIOLOGY in the FIELD OF MOLECULAR BIOLOGY,

SCHOOL OF BIOLOGICAL SCIENCES

Preference will be given to applicants with experience in recombinant DNA techniques, for example vector construction involving either E coli or S cerevisiae plasmids, and DNA sequencing. Good prospects exist for collaborative research at the molecular level with existing research groups within the school currently working in plant physiology/biochemistry, virology (particularly of the Herpes group), developmental biology of Dictyostelium and X-inactivation of marsupials. The CSIRO Plant Physiology Unit of the Division of Food Research is also located in the School and regularly participates in collaborative projects.

The successful candidate will be expected to assist in the teaching of undergraduate courses (mainly at the upper level) and to supervise postgraduate research students. Further information about the position can be obtained from Professor D W Cooper, Head of the School of Biological Sciences.

LECTURER IN PHYSICS, SCHOOL OF MATHEMATICS AND PHYSICS (which also includes ELECTRONICS and COMPUTING)

The duties of the appointee will include teaching in physics to honours degree level (some teaching in electronics may also be required) and conduct of research in Physics including postgraduate research supervision. The field of research interest of applicants is not restricted, but some preference may be shown for physicists with strong theoretical backgrounds who are interested in interaction with experimentalists working in the areas of optical physics, atomic and molecular physics, physics of materials (including thin films) and other areas of interest in the discipline.

June 1984. A J T Ford, Registrar. (W1088)A

THE UNIVERSITY OF AUCKLAND NEW ZEALAND

DEPARTMENT OF PSYCHIATRY AND BEHAVIOURAL SCIENCE

AN ASSOCIATE-PROFESSORSHIP IN BEHAVIOURAL SCIENCE

Closing date: 1 October 1984. This position, which has been created to head the Behavioural Science Section within the newly combined Department, involves the organisation and administration of the five year Behavioural Science teaching programme within the present medical course. Candidates should hold a doctoral level qualification in a Behavioural Science discipline such as Psychology or Sociology. Preference will be given to those with experience of teaching Behavioural Science in a medical context, and experience of teaching Behavioural Science in clinical settings would be an advantage. The successful applicant will be expected to undertake substantive research work in Behavioural Science or Behavioural Medicine and organisational or leadership experience would be desirable.

DEPARTMENT OF PHARMACOLOGY AND CLINICAL PHARMACOLOGY

A LECTURESHIP/SENIOR LECTURESHIP (NON-MEDICAL) IN PHARMACOLOGY

Closing date: 31st August 1984. Candidates must hold a PhD degree in pharmacology, physiology, biochemistry or a related discipline. Preference will be given to candidates with experience in teaching in practical classes in Science pharmacology. Candidates with experience in teaching in practical classes in Science pharmacology. Candidates should have proven ability to teach and conduct independent research. Preference may be given to those with a research interest in one of the following areas: pharmacokinetics, drug metabolism, applied cardiac or autonomic pharmacology or neuropharmacology, adenosine pharmacology or metabolism.

Commencing salaries will be established within the appropriate scale. Present scales are: Associate Professor is NZ$37,896 per annum; Senior Lecturers NZ$27,088-$30,127 per annum; and Lecturers NZ$21,660-S$25,684 per annum. Salaries are currently supplemented by an $8 week cost-of-living allowance. Conditions of Appointment and Method of Application are available from the Assistant Registrar (Academic Appointments), University of Auckland, Private Bag, Auckland, New Zealand, or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications in accordance with the Method of Application should be forwarded as soon as possible but not later than the closing dates stated. (W1085)A

BIOTECHNOLOGY

Celltech is one of the World's leading specialist biotechnology companies. Based in the UK. The Company is backed by four major City of London financial institutions. A strong scientific team has now been built up in the areas of recombinant-DNA and cell-hybridisation and continued expansion is envisaged; close links are also maintained with the academic community. The Company aims to exploit the potential of discoveries arising in the field of biotechnology, and commercial successes have already been achieved in several areas.

MOLECULAR BIOLOGISTS

We require post-doctoral molecular biologists (or graduates expecting to obtain a PhD this year) to join our molecular biology and immunology divisions. The successful candidates will be involved in the cloning and sequencing of genes coding for commercially important proteins. Expertise in isolation and handling nucleic acids is desirable. Previous experience in the preparation and cloning of cDNA will be an advantage.

Please quote reference number 205.

MOLECULAR GENETICS

A position is available in the Molecular Genetics Department for a post-doctoral Scientist to join a group developing novel mammalian gene expression systems. Experience with viral vectors or amplifiable genes is desirable.

Please quote reference number 206.

GROUP LEADER

We are currently seeking an experienced person to lead our protein microsequencing facility. The post will involve research into sequencing methods, interactions with recombinant-DNA project teams and an involvement in downstream processing and quality control.

Please quote reference number 207.

TECHNICIAN IN HYBRIDOMA Immunology

We are currently seeking a technician to make monoclonal antibodies within our Hybridoma group.

Experience in tissue culture is essential and knowledge of serological assays and fusion techniques would be advantageous.

This position would suit an HNC/HND or first degree applicant.

Please quote reference number 208.

To apply, please send a full C.V. to Jane Walley, Personnel Officer, Celltech Limited, 244 Bath Road, Slough, Berks. SL1 4DY quoting the appropriate reference number.

(3078)A

CELLTECH

IRI

Inveresk Research International

MASS SPECTROMETRY

Inveresk Research International Limited, an expanding contract research organisation, now has the following vacancy within its area of Analytical Chemistry:—

RESEARCH OFFICER/ MASS SPECTROMETRY

This vacancy is for a chemist conversant with the use of mass spectrometry in the analysis of biological samples. Applications include the development of GC and HPLC separation techniques and the identification and measurement of drugs and metabolites in body fluids.

The successful candidate will assume responsibility for a Finnigan 4500 mass spectrometer and data system, and will liaise with research groups in metabolism and chemistry.

Applicants should have a PhD in applied mass spectrometry in the biological sciences or should be graduates with similar experience.

Salary will be on the scale £7,599 to £9,033 or £8,811 to £10,485, with placement depending upon qualifications and relevant experience. In addition, the Company offers modern staff benefit schemes, including free private health care, and pleasant working conditions in a rural setting close to Edinburgh. Relocation assistance may also be provided, where appropriate.

Full details in writing should be forwarded to the Personnel Department, Inveresk Research International Limited, Inveresk Gate, Musselburgh, quoting reference 1440.

(W1087)A

PUBLIC HEALTH LABORATORY SERVICE BOARD

PHLS Centre for Applied Microbiology & Research

Microbial Technology Laboratory

MOLECULAR GENETICIST

A Molecular Geneticist is required to join a group in the Microbial Technology Laboratory concerned with all aspects of cloning and gene expression. Systems under investigation include the cloning of bacterial genes of potential commercial interest, the sequencing of viral genomes and the cloning of eukaryotic genes.

Candidates should have an appropriate honours degree and post graduate experience in Molecular Genetics with preferably some experience in the cloning and expression of eukaryotic genes.

Salary will be either in the Basic Grade £6,277 — £8,349 or Senior Grade £9,010 — £11,649 Microbiologist scale as appropriate. National Health Service terms and conditions will apply.

Further details on the nature of this post can be obtained by contacting Dr N Minton, Microbial Technology Laboratory, CAMR, Telephone 0980 610391.

Application forms can be obtained from Mrs M Bushby, Personnel Officer, CAMR, Porton Down, Salisbury SP4 0JG and should be returned together with a curriculum vitae and the name of two referees. Closing date will be 13th July 1984.

(2994)A

NORTH CALIFORNIA OCCUPATIONAL HEALTH CENTER

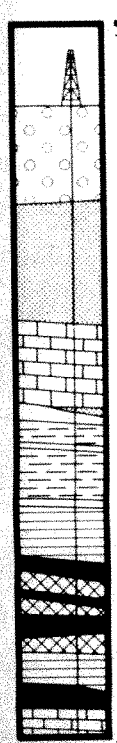
SENIOR RESEARCH TOXICOLOGIST

The Northern California Occupational Health Center is seeking a Senior Research Toxicologist with expertise in research methodology, and basic and applied toxicology. The individual may hold an MD or a PhD degree and have demonstrated ability to obtain basic research grants in her/his specialized area of occupational toxicology research. The individual should be anxious to work in collaboration with physicians working in a major occupational health program at UCSF School of Medicine with special expertise preferably in heavy metals or nephro-toxicology, or in occupational cancer, or neurotoxicology.

The University of California is an affirmative action employer which does not discriminate on the basis of race, sex, or age.

For inquiries please call or write: Charles E Becker, M D, Professor of Medicine, UCSF, Head, Divsio[illegible] Occupational Medicine and T[illegible]cology, San Francisco General Medical Center, 1001 Potrero Avenue, Bldg 30, 5th Floor, San Francisco, CA 94110; (415) 821-5200.

Applications must be received by August 10, 1984. (NW642)A



Micropalaeontologist

Based Aberdeen

We currently have an interesting opportunity within our Stratigraphic Laboratory in Aberdeen for an applied micropalaeontologist (specialising in Foraminifers).

The post will ideally suit candidates, with up to 3 years' post doctoral industrial/ academic experience, who are looking for a challenging appointment with close multi-disciplinary interaction.

The successful candidate will join a team of BP biostratigraphers who currently operate in Canton (China), Aberdeen and in the BP Research Centre, Sunbury-on-Thames, Middlesex.

An attractive remuneration package includes a non-contributory pension scheme, subsidised restaurant, on-site sports and social facilities and relocation assistance where appropriate.

Please write in detail, quoting reference SR 2618 to: Anne Hendry, The British Petroleum Company plc, BP Research Centre, Chertsey Road, Sunbury-on-Thames, Middlesex.

(3097)A

BP **BP Exploration Limited**

UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST THOMAS'S HOSPITALS

UNIT FOR METABOLIC MEDICINE, DEPARTMENT OF MEDICINE, GUY'S HOSPITAL MEDICAL SCHOOL

MEDICAL LABORATORY SCIENTIFIC OFFICER

Applications are invited from recent graduates in Biochemistry or Biological Sciences for a two-year funded post within the Unit for Metabolic Medicine (Director, Professor H Keen). The appointee will join a small group working on the macrovascular complications of Diabetes Mellitus and will play an important role in a current study investigating the efficacy of hypolipidaemic drug therapy in dyslipidaemia. The work involves lipid and lipoproteia separation and analysis using ultracentrifugation, enzymatic autoanalysis and immunological methods and includes technical assistance at patient clinics.

Salary during the first year of appointment according to age on scale £4,674—£4,818 per annum, rising after one year's satisfactory service to £5,491 per annum, plus £1,042 per annum London Weighting (salary scales under revision).

Applications, including age, qualifications, experience and the names of two referees, should be addressed to The Secretary, Guy's Hospital Medical School, London Bridge, London SE1 9RT, to arrive by 12th July, 1984, and quoting Ref. UMM1. (3010)A

MONASH UNIVERSITY
Melbourne, Australia

CENTRE FOR EARLY HUMAN DEVELOPMENT

DIRECTOR
FACULTY OF MEDICINE

The University Council wishes to appoint a Director to the Centre for Early Human Development following the appointment of the present Director, Dr J E Maloney, to a position at the University of Calgary. The position will be vacant as from 1 July 1984.

The Centre is situated at the Queen Victoria Medical Centre together with the University Departments of Obstetrics and Gynaecology, and Paediatrics. A new hospital with 436 beds is under construction at Clayton adjacent to Monash University and it is expected that it will be occupied by the Queen Victoria Medical Centre, the associated University Departments, and the Centre for Early Human Development OSC is approximately 478 square metres, excluding space shared with the University Departments.

The Centre for Early Human Development was established in 1978 to undertake research on prenatal and postnatal development and at present comprises an established multidisciplinary team working on aspects of cardiorespiratory physiology in the period surrounding birth. The new Director would be required to assure the continuance of this work and to seek to develop other aspects of research in early human development. Opportunities exist for collaboration with Departments of the University, both at Queen Victoria Medical Centre and at the adjacent main campus of the University, and with Departments of the Medical Centre. A medically qualified Director will have the opportunity of undertaking clinical responsibility in his field of expertise.

The appointment is for five years in the first instance. Enquiries about the Centre and the Faculty of Medicine may be directed to Professor G C Schofield, Dean of the Faculty of Medicine, Monash University.

Salary $A38,085 — $A51,006 per annum, depending on qualifications and experience. A loading will be paid to a medically qualified appointee. Provision for superannuation, outside studies programmes, travel and removal allowance, and temporary housing assistance.

Information on application procedure and further particulars may be obtained from the Registrar, Monash University, Clayton, Victoria 3168, Australia, or the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications should reach the Registrar not later than 15 August 1984. Council reserves the right to make no appointment or to appoint by invitation at any stage.

An equal opportunity employer.
(W1090)A

UNIVERSITY OF EDINBURGH

FACULTY OF VETERINARY MEDICINE

DEPARTMENT OF VETERINARY PATHOLOGY

Applications are invited from veterinary graduates for the post of lecturer in Pathology.

The Department has an integrated approach to research and teaching in all aspects of Pathology (immunology, microbiology, morphological pathology) and has considerable expertise and facilities for work in these areas. The person appointed will be expected to contribute to teaching in pathology, to share the service commitments in diagnostic pathology (including post mortem work) and to undertake further research training in pathology leading to a higher degree (PhD or MRC Path if this has not already been obtained).

The salary scale is £7,190 — £14,125 but initial appointment will not exceed £8,975 with first increment on October 1st 1984.

Six copies of application with the names of 3 referees should be submitted to the Secretary to the University, Old College, South Bridge, Edinburgh from whom further particulars can be obtained. Closing date for applications 13th July, 1984. Please quote Reference No.1140.
(2909)A

PRINCESS MARGARET CHILDREN'S MEDICAL RESEARCH FOUNDATION

Perth, Western Australia

CLINICAL IMMUNOLOGIST

(Joint Clinical and Research Appointment)

The Princess Margaret Children's Medical Research Foundation (CMRF) seeks suitably qualified applicants for a joint appointment to the Foundation's Clinical Immunology Research Unit (CIRU) and the Princess Margaret Hospital's (PMH) Clinical Staff.

Applicants are sought with F.R.A.C.P. or equivalent qualification, preferably in Paediatrics, and research experience in Clinical Immunology.

The salary level and status of the appointee will be commensurate with their joint responsibilities (1) to the Director of the CIRU as Research Fellow/Senior Research Fellow for research responsibilities within the Unit (conditions as per NH & MRC, plus clinical loading) and (2) to the Medical Director as Clinical Staff Specialist for Clinical and Laboratory service responsibilities (salary in accordance with the West Australian Metropolitan Teaching Hospitals Agreement 1978 — Clinical Staffs). For further information contact Professor K J Turner (Director, CIRU) by telephoning (09) 382 8592 or Dr D R Fry (Medical Director, PMH) on (09) 382 8241.

The appointment will be for an initial period of three years, after which the basis for renewal or permanence will be determined. Applications, including curriculum vitae and the names and addresses of three referees should be submitted to **Mr K Williamson, Secretary, CMRF, GPO Box D184, Perth, W.A. 6001, by August 31st, 1984.**

(W1080)A

Experimental Biologist

Helminth Chemotherapy

Starting salary in the range £8,700 — £10,700
Beckenham

The Wellcome Foundation Limited is a major group of pharmaceutical and chemical companies with headquarters in the UK and a turnover close to £700m.

Our Department of Biochemical Parasitology at Beckenham now requires an experienced research Biologist to join its strong multidisciplinary team of biochemists, physiologists, chemists and parasitologists engaged in the research and development of new medical and veterinary anthelmintics. You will be expected to design and operate assays for the evaluation of anthelmintics and to undertake independent research within the framework of our research programme.

A first degree in Biological Science and a PhD in parasitology are essential, possibly with 1-2 years' post doctoral research experience. You will have a keen interest in the field of chemotherapy, and will enjoy both interacting in a team and independent work.

Appointment will be for a period of 12 months in the first instance with chance of renewal.

Excellent benefits include 5 weeks' holiday, pension and life assurance scheme, subsidised canteen and first class sports and social facilities.

Please write with full cv, quoting ref WRL/ 284, to Dr H D Avshu, Personnel Officer, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.
(3098)A



Wellcome

Public Service Commission of Canada / Commission de la Fonction publique du Canada

Scientific Advisors, Toxicology

Health and Welfare Canada
Health Protection Branch
Ottawa, Ontario

We require experienced senior toxicologists to fill several positions in the Toxicological Evaluation Division. We will expect you to validate, review and evaluate reports on a range of toxicity studies on chemicals in the food supply, such as pesticides, food additives and chemical contaminants. You will become involved in the assessment of potential hazards and risks to public health. Employment in the division offers unique opportunities to gain diversified toxicological advisory experience in a government setting and to have input in the extensive research program.

You require a university degree with specialization in a biological science such as toxicology, pharmacology, biochemistry, with experience in laboratory animal toxicity studies and bioassays. In addition, you need experience in reviewing and evaluating reports on toxicity studies and in conducting health hazard and risk assessments. Some positions also require additional experience in preparing recommendations based on toxicological evaluations and health hazard assessments. Language requirements vary according to the position being filled.

We offer a salary ranging from $31,791 to $48,935 commensurate with your qualifications and experience.

Forward your résumé and/or application form, quoting reference number S-84-31-0293-47JG-(G26), to:
J. Girling (613) 993-5331
Public Service Commission of Canada
Ottawa, Ontario K1A 0M7

Closing Date: 20 July 1984
Tout renseignement est disponible en français en s'adressant à la personne susmentionnée.

(NW634)A

The Public Service of Canada is an equal opportunity employer Canada

DIRECTOR

Goodwin Institute for Cancer Research

Applications are invited for the position of director. This chief executive scientist, reporting to a Board of Trustees, is responsible for the development of research programs, supervision of operations, and governmental and industrial relations associated with grants, contracts, and fund raising. The director is encouraged to conduct personal research.

The Institute, chartered in 1959 as a private, non-profit medical research corporation, moved into 30,000 square feet of new, well-equipped laboratories in 1980. The building also houses a Primary Genetic Center for specific pathogen-free rodents operated under contract with the National Cancer Institute. Current staff numbers 40 persons and annual budget is $2 million.

Candidates should have a Ph.D., D.V.M., or M.D. and be qualified to apply current biotechnology to studies of the molecular nature of experimental cancer induction, growth, and control. Administrative experience is essential, publications and acquired support are desirable, and imagination is useful.

Appy to: Search Committee, Goodwin Institute for Cancer Research, 1850 Northwest 69 Avenue, Plantation, FLa. 33313.

(NW629)A

WASHINGTON UNIVERSITY
DEPARTMENT OF CHEMISTRY
CHAIRMANSHIP

Applications are invited for the position of Chairman of the Department of Chemistry at Washington University, St Louis, MO. Applicants should have a strong research program, and proven teaching and administrative experience.

Interested scientists should submit a complete curriculum vitae and a list of at least three individuals who can be contacted for references to: Chairman, Chemistry Search Committee, Box 8094, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, MO 63110.

Washington University is an Equal Opportunity Employer M/F/N/V

(NW626)A

IMPERIAL COLLEGE
NORTH SEA OIL RESEARCH

Applications are invited for an expanding programme of research into reservoir and recovery problems related to North Sea oil fields. Industry sponsored post graduate and post doctoral posts are available.

The research involves theoretical and experimental investigations into the flow of fluids through porous media and model networks.

Enquiries to Dr. Richard Dawe, Mineral Resources Engineering Department, Imperial College, London SW7 2BP. Tel: 01-589 5111 Ext. 1555 or 1537. (3018)A

UNIVERSITY OF PENNSYLVANIA
SCHOOL OF VETERINARY MEDICINE
CHAIR OF THE DEPARTMENT

The University of Pennsylvania is inviting applications and nominations for the Chair of the Department of Clinical Studies (Philadelphia) in the School of Veterinary Medicine.

Duties are the fostering of creative scholarship, teaching and clinical training, and leadership of the faculty not only in traditional university pursuits but also in the operation of a major urban teaching hospital. This Chair offers an unexcelled opportunity for academic leadership in clinical veterinary medicine. Nominations may be informal.

Applications should include a curriculum vitae and the names of three referees.

Communications should be addressed by Sept 30, 1984, to Dr D S Kronfeld, School of Veterinary Medicine, University of Pennsylvania, 382 West Street Road, Kennett Square, PA 19348.

The University of Pennsylvania is an equal opportunity, affirmative action employer (NW632)A

NEW YORK MEDICAL COLLEGE
DEPARTMENT OF MEDICINE
POSTDOCTORAL POSITION IN THE AREA OF MOLECULAR BIOLOGY

To study the regulation of mammalian liver cytochome P450 heme matobolism. Experience in purification, enzymology, biochemistry or related biological science desired.

Salary range: $18,000 to $20,000 depending on capability and past experience. 1 year appointment available now with possibility of 2 to 3 year extensions.

Send curriculum vitae, brief description of research interest and names of two references to: N G Ibraham, PhD, Department of Medicine, New York Medical College, Valhalla, NY 10595

(NW631)A

UNIVERSITY OF READING
SUB-DEPARTMENT OF HORTICULTURE
GRADUATE RESEARCH OFFICER

required for fixed period of one year to carry out investigations on horticultural products under the supervision of the Professor of Horticulture.

Applicants should be graduates in plant sciences, botany, horticulture or other relevant science. Salary up to £6745 p.a. (under review).

Apply quoting ref. R27A for application form to Personnel Officer, University of Reading, Whiteknights, Reading RG6 2AH.

(3013)A

THE ANIMAL VIRUS RESEARCH INSTITUTE
HEAD OF MOLECULAR BIOLOGY

Applications are invited for a new post of Head of the Division of Molecular Biology, the appointment to commence 1 October 1984. The successful candidate would also become Head of the Department of Biochemistry, and would lead a vigorous team investigating the biochemistry and genetics of several important exotic animal diseases including foot-and-mouth disease, bluetongue, and African swine fever viruses.

Candidates should have a high academic research record in the molecular biology of viruses and proven skills of leadership.

Salary in scale of £16,343-£20,794 (Grade 6 previously known as Senior Principal Scientific Officer). Sick leave and non-contributory superannuation schemes. 25 days annual holiday. House may be available for suitable married applicant. The successful applicant may qualify for financial assistance with removal expenses. The AVRI is an equal opportunity employer.

Application forms from the Secretary, Animal Virus Research Institute, Pibright, Woking, Surrey GU24 0NF. Tel: 0483-232441. Closing date for applications 10th July, 1984. (2966)A

UNIVERSITY OF WALES WELSH NATIONAL SCHOOL OF MEDICINE

DEPARTMENT OF MEDICAL BIOCHEMISTRY

RESEARCH OFFICER

Applications are invited for the post of Research Officer to work for two years on a project investigating the role of oxygen radical mediated protein modification in the pathogenesis of rheumatoid arthritis. The post is based in the School's Department of Medical Biochemistry and will involve close collaboration with the Rheumatology Unit. Candidates should have a degree in Biochemistry or a related discipline.

Commencing salary £6,310 per annum on the scale for University Research and Analogous Staff Grade 1B (£6,310 — £8,530 per annum).

Further particulars (quoting Ref. No. M6/116) available from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN (Tel. No. (0222) 755944 Ext. 2296) to whom applications in the form of a curriculum vitae with the names and addresses of two referees should be sent no later than 3 weeks after the date of the appearance of this advertisement. (3017)A

PLANT TISSUE CULTURE

A position is available at the Tissue Culture Laboratory of a horticultural company in West Chicago, Illinois. The qualifications for the position are: MS or BS in horticulture or related area with a strong background and interest in plant tissue, cell and protoplast culture.

Send résumé to: Dr Irwin Chu, George J Ball, Inc, P O Box 335, West Chicago, IL 60185.

(NW635)A

THE UNIVERSITY COLLEGE OF WALES ABERYSTWYTH POSTDOCTORAL RESEARCH: MICROBIAL PHYSIOLOGIST

Applications are invited for a Postdoctoral post funded by the SERC Biotechnology Directorate, and concerned with biotransformations by species of Clostridium. The applicant will join an established research group led by Professor J G Morris, studying fermentation processes in anaerobic bacteria maintained in batch and continuous flow culture. Informal enquiries may be made of Dr Douglas Kell by telephoning (0970) 3111, Ext 3055.

The appointment will be for up to 3 years, depending on the starting date, and will commence not later than 1 October 1984. The salary scale is £7190 to £11615 per annum (under review).

Application forms and further particulars may be obtained from the Staffing Officer, The University College of Wales, Old College, King Street, Aberystwyth, SY23 2AX (Tel (0970) 3177, Ext 207).Completed applications to be returned as soon as possible. (3011)A

The Department of Zoology of the British Museum (Natural History), has in its charge the national collections of all recent animal groups other than the Insecta. Its pre-eminent position as a centre for research in systematic zoology springs from the immense scale and diversity of these collections (enormously rich in type and other historically important material), and their provenance, and from the special responsibilities and opportunities which derive from the Museum's national and international obligations in science.

Supported by two Deputy Keepers, the successful candidate will control and co-ordinate the work of some 96 staff, including 87 scientists, responsible for the care, maintenance and documentation of the collections in their charge, and research and the provision of identification and advisory services. Senior members of staff are authorities of international standing in their fields, and, exploiting a range of techniques, their research work is concerned principally with descriptive and revisionary taxonomy, the elucidation of phylogenetic relationships and the study of evolutionary patterns and processes.

Keeper of Zoology

As a scientist of high professional standing, the Keeper will provide academic leadership to the Department and will have the capacity to motivate a highly productive staff force to further success. By keeping abreast of current scientific developments, he/she will formulate and develop research policies and will play an important role in a 'collegiate' system of top management which, while preserving the ultimate authority of the Trustees and the Director, involves Heads of Departments in forming and sharing responsibility for policy decisions which set the course for the whole Museum.

Candidates should normally have a good honours degree or equivalent (including overseas) qualification in Zoology or an allied discipline, and experience in the development, management and direction of research programmes.

Appointment will be as Grade 6 (formerly Senior Principal Scientific Officer), with starting salary (including £1250 Inner London Weighting) £16,855 rising to £22,040. SALARIES UNDER REVIEW.

For further details and an application form (to be returned by 20 July 1984) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). **Please quote ref: S/6249.**

(3075)A

The Civil Service is an equal opportunity employer

British Museum (Natural History)

SPECTROSCOPIST

The California Institute of Technology is seeking an NMR Spectroscopist for the NSF Southern California Regional NMR facility, beginning in August. The position involves aiding the Manager in daily operation, NMR methods core research & instrumentation development, and collaborative projects with users. The facility has a Bruker WM-500 and a home built 200 MHZ solids spectrometer and is staffed with student operators & electronics technician. PhD in Chemistry or related discipline & research experience in NMR spectroscopy required. Must have command of theory and applications of modern NMR spectroscopy, including 2-D.

Qualified applicants are encouraged to send a résumé with salary history to Supervisor of Employment, Position No 229, Caltech, Pasadena, CA 91125.

MRC BRAIN METABOLISM UNIT
Edinburgh
Organic Chemist

The MRC Brain Metabolism Unit invites applications from suitably qualified chemists for the post of Organic Chemist with special expertise in the synthesis of neuropeptides and appropriate analogues. Candidates of immediate post-doctoral or equivalent status will be preferred. A period (minimum 3 months) of training in the peptide chemistry in the laboratory of Dr R.C. Sheppard (MRC Laboratory of Molecular Biology) may be offered to a suitable candidate who, although highly competent in organic chemistry, has no special expertise in peptide chemistry.

The person appointed will work in close association with the Molecular Biologists in the Unit who are interested in the mechanisms of synthesis, release and mechanism of action of neuropeptides in mammalian brain. The appointment will be short-term (3 years). Remuneration will be at an appropriate point on the scales for university non-clinical academic staff.

Further details can be obtained from the Director, Dr G. Fink, MRC Brain Metabolism Unit, Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ. Four copies of applications and the names and addresses of three referees to be sent to Mrs A. Milligan, Administrative Officer, MRC Brain Metabolism Unit, Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ by Friday, July 27. (2917)A

ICI

CORPORATE BIOSCIENCE GROUP

PLANT MOLECULAR BIOLOGY

POST DOCTORAL RESEARCH FELLOW AND GRADUATE RESEARCH SCIENTIST

Two vacancies have arisen in the Plant Molecular Biology Group at ICI's Runcorn Laboratories. The successful candidates will join a small team investigating plant gene structure and function. This group is part of a large molecular biology and biochemistry team with long term research goals in mammalian and plant biology.

The successful applicants will join established programmes working on the isolation and characterisation of phenylalanine-amonia lyase genes from French bean, and polygalacturonase genes from ripening tomatoes. The post-doctoral fellow is expected to have experience in cloning genomic DNA, DNA sequence analysis, s_1-mapping etc. The research scientist should have a sound practical knowledge of modern molecular biology techniques. Both positions are available immediately. The starting salary for the graduate will be minimum of £7,000 plus benefits. The position of post doctoral research fellow is initially available for a 2 year period and the starting salary will not be less than £9,500 depending upon experience.

Applications including a CV please to Miss E H Lauermann, Personnel Officer, PO Box No. 11, The Heath, Runcorn, Cheshire by 26 July, 1984. (3004)A

FERMENTATION CONSULTANT

We are a rapidly growing biotechnology firm manufacturing and selling enzymes and other process aids to the food and drink industries worldwide. Our manufacturing facilities are located in Western Europe and we are currently engaged in expanding our fermentation capacity substantially. We are seeking the services of an experienced fermentation technologist to act as consultant.

The responsibilities of this consultancy will be two-fold (1) to oversee/advise on the commissioning of our new plant and (2) to contribute technically at the pilot and production scale to the optimisation of microbial (bacterial and fungal) fermentation processes currently under development.

Application and correspondence should be submitted to the undersigned below and will be treated in strictest confidence. Keith Walesby, Bio/ Technology, 4, Little Essex Street, London, UK, WC2R 3LF.

(W10078)A

INSTITUTE OF CANCER RESEARCH

The Institute of Cancer Research has the following technical vacancies in its Drug Development Section based at Sutton, Surrey.

RESEARCH OFFICER

The successful candidate will join a small team studying the mechanism of action and general pharmacology of novel antitumour agents. Candidates with a degree or equivalent (MIBiol) in the biological sciences, particularly in biochemistry and pharmacology, are invited to apply. Salary in range £6,204 – £8,126 p.a. plus London Allowance of £623 p.a. **Ref 301/ B/ 49.**

TECHNICIAN

The successful candidate will join a small team studying the mechanism of action and general pharmacology of novel antitumour agents. Facilities are available for further study and the successful applicant will be encouraged to exploit these. Candidates with an HNC or equivalent in the biological sciences, particularly in biochemistry and pharmacology, are invited to apply. Salary in range £5,491 – £6,436 p.a. plus London Allowance of £623 p.a. **Ref. 301/ B/ 50.**

Technician

This vacancy is for an Organic Chemistry Technician. The successful candidate will be invited to join a small multi-disciplinary group concerned with the synthesis and evaluation of quinazoline-based antifolates as potential cancer chemotherapeutic agents. Applicants should possess a degree or equivalent and preferably laboratory experience in general heterocyclic organic chemistry. Salary in range £5,491 – £6,436 p.a. plus London Allowance of £623 p.a. **Ref. 301/ B/ 51.**

Applications for any of the above posts should be sent in duplicate and giving the names, addresses and telephone numbers of two referees to* the Personnel Officer, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, *and quoting the appropriate reference number. (3059)A

Deputy Director General

INTERNATIONAL COOPERATION

The International Center for Agricultural Research in the Dry Areas (ICARDA) with headquarters in Aleppo, Syria, invites applications for the Position of Deputy Director General – International Cooperation.

Responsibilities: Responsible directly to the Director General for:
1. Developing policies on matters relating to the Center's international relationships.
2. Establishing and strengthening collaborative links with national agricultural research and training programs in the region (Morocco and Pakistan) and where appropriate, in less developed countries elsewhere in the world.
3. Developing research and training linkages with regional and international organizations and centers and with appropriate resource institutions in advanced countries.
4. Establishing and monitoring the execution of cooperative agricultural research and training projects.
5. Supervising the regional offices of ICARDA (currently Cairo, Tunis, Amman, Beirut).
6. Assisting the Director General in contacts with Governments in the region and with donor organisations.

Qualifications:
1. Ph.D. degree in an appropriate field of agricultural research.
2. A minimum of five years in research leadership position.
3. Wide international experience and proven leadership ability.
4. A good knowledge of the Near East and North Africa region.
5. Fluent in spoken and written English essential. Knowledge of Arabic and/or French very desirable.

Conditions of appointment:
International tax-free salary based on background and experience, use of car, allowance towards housing, annual home leave, non-contributory medical insurance scheme, pension fund (TIAA-CREF in USA).
Candidates should send two copies of their curriculum vitae and names of three referees by airmail quoting reference DIR/1/84 to:

Dr. Mohamed A. Nour
Director General, ICARDA
P.O. Box 5466, Aleppo, Syria. (W1084)A

McGILL UNIVERSITY

MOLECULAR GENETICS GROUP

The Department of Biochemistry, Faculty of Medicine, McGill University is seeking three or four highly qualified individuals to form a research group with interests in the applications of molecular genetics to medically relevant topics. All positions are tenure track at the level of assistant or associate professor. Candidates should have a minimum of two years postdoctoral experience and demonstrated excellence in the application of recombinant DNA technology to animal cell systems. Each new member is expected to develop a separate, vigorous and competitive research program. Positions will be tenable after April 1, 1985.

Applicants are invited to send a complete curriculum vitae, copies of recent publications and the names of three references to Dr. C.P. Stanners, Department of Biochemistry, McGill University, 3655 Drummond Street, Montreal, Quebec, Canada H3G 1Y6.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada; however others are encouraged to apply. (NW612)A

HEAD OF THE UNIT OF EQUINE PATHOLOGY AT THE ANIMAL HEALTH TRUST, NEWMARKET

A veterinary pathologist with relevant post graduate qualifications, preferably with experience in equine pathology, is required. The selected applicant will be expected to coordinate routine pathology, organise specific investigations in relation to disease incidence and undertake collaborative research. Good facilities are availble and excellent supporting staff are in post. The position offers good opportunities for an enthusiastic and progressive approach.

Salary range £13,515 to £16,925. Further details from the Director, Animal Health Trust, Balaton Lodge, Snailwell Road, Newmarket, Suffolk CB8 7DW. Applications including a detailed cv and list of publications should be submitted before August 11, 1984. (2986)A

METEOROLOGICAL OFFICE AND OXFORD UNIVERSITY

THE ROBERT HOOKE INSTITUTE FOR ATMOSPHERIC RESEARCH

SCIENTIFIC COMPUTING SPECIALIST

The Department of Atmospheric Physics, Oxford University, and the Meteorological Office, are forming a joint institute for atmospheric research to be named the Robert Hooke Institute. The work of the Institute will include satellite meteorology, atmospheric/ocean modelling for climate research and advanced instrument development and data analysis for space projects of various kinds. It will be equipped with computing facilities based on PDP 11/70 and 2 VAX 11/750 processors with image display and other advanced peripherals, and with access to mainframes at the University computing centres of Oxford, London and Manchester and to the IBM 3081/CYBER 205 at Bracknell.

The creation of the Institute provides an opening for a computer specialist interested in joining a lively scientific organization in the forefront of research using measurements from space. The successful applicant will be expected primarily to manage and develop computing facilities for the Institute's research programmes, particularly satellite meteorology, but he or she will also be encouraged to participate in the research itself. Experience with DEC hardware and software would be a considerable advantage.

The post is on the University Research Support grade 1A scale, carrying a salary in the range £7,190-£11,615 p.a. plus membership of the USS pension scheme. The appointment will commence on 16 July 1984 or as soon thereafter as is convenient.

Please apply with curriculum vitae and the names and addresses of two referees to: Dr. F.W. Taylor, Department of Atmospheric Physics, Clarendon Laboratory, Oxford OX1 3PU. (3016)A

Research Biochemist

Opportunity for a young graduate

Roche Products Limited, part of F Hoffman-La Roche Company AG, and a world leading research-based pharmaceutical company, wish to strengthen their team working on rheumatic and inflammatory diseases. This has produced an excellent career opportunity for a young biochemistry graduate to work in the Applied Biology Department.

As part of a small multi-disciplinary team, the successful candidate will be working in a modern laboratory within a new special purpose research building – offering an excellent environment in which to develop individual skills and pursue a career in advanced research.

To qualify applicants must be recent first degree graduates with an active interest in the control of cell growth and development. Exposure to the techniques used in protein purification would be particularly advantageous.

In return we offer an attractive salary and benefits to be expected of a world leading pharmaceutical company.

Please send curriculum vitae to **Mrs M.L. Clewley, Personnel Officer**, or telephone **Mrs D.M. Turner, Welwyn Garden City (07073) 28128 ext. 2129**, for an application form.

Roche Products Ltd., PO Box 8, Welwyn Garden City, Herts. AL7 3AY.

ROCHE

(3023)A

Wheat Breeder

Hybrid Parent Development

Nickerson RPB Limited

An experienced wheat breeder is required to initiate work on the development of parents suitable for F1 hybrid wheat production. This new challenging position requires an imaginative breeder who is willing to work on his own initiative, but closely with other breeders and breeding support teams as part of a small team. The location is at Rothwell, Lincolnshire, but candidates must be willing to travel, particularly within Europe. Knowledge of one or more European languages would be an advantage.

The successful candidate is likely to have a BSc or PhD and be currently involved in wheat breeding or closely related work. Fresh graduates need not apply.

Salary and conditions of employment will be commensurate with responsibility as you would expect of a major commercial breeding company.

Please write or telephone for an Application Form to :-

The Group Personnel Department
The Nickerson Seed Company Limited
The Joseph Nickerson Research Centre
Rothwell
LINCOLN LN7 6DT. Tel: Swallow 471

The Nickerson Seed Company Ltd

(3043)A

At the School of Sciences at the *Technische Universität Braun-schweig (Braunschweig Institute of Technology)* and at the *Gesellschaft für Biotechnologische Forschung GmbH in Braunschweig-Stöckheim (Institute for Biotechnological Research, GBF)* four positions are open for *professorships* in the fields of:

1. **Biological process engineering**
2. **Microbiology**
3. **Enzyme Technology**
4. **Genetics**

The successful applicants for each of these positions will be offered a chair as Professor at the Technische Universität Braun-schweig and then will be delegated to the GBF to become head of a department. The GBF is a large research center, supported by the Federal Government.

The heads of departments in the GBF will be expected to work in one independent research field and shape the GBF's joint research and development programmes. The TU Braunschweig expects active participation in the new programmes of biotechnology and biological process engineering.

Applicants must have a Ph.D. in Science or Engineering and should be highly qualified in one of the above noted fields, documented by a distinct research record and teaching experience.

Applications should be sent to the following address not later than August 15th, 1984:

Ber Vorsitzende der Naturwissenschaftlichen Fakultät der TU Braunschweig, Pockelsstraße 14, D-3300 Braunschweig,

who will give further information on special request.

(W1079)A

PROFESSOR OF BIOSTATISTICS

HARVARD SCHOOL OF PUBLIC HEALTH

This position is to lead and enlarge the Department of Biostatistics' current research and teaching program in statistics and the environment. Candidates should have an outstanding research record in biostatistics and an interest in problems of the environment and health. Candidates should have made significant contributions to statistical methodology. Strong teaching credentials are essential.

Applications from qualified minority and female candidates are particularly encouraged.

Candidates should send a curriculum vitae, statement of current and future interests, and the names of at least three individuals who would be willing to respond to a request for evaluation to:

Professor Donald F. Hornig
Chairman, Search Committee
Harvard School of Public Health
665 Huntington Avenue, Room 1308
Boston, Massachusetts 02115, USA

(NW639)A

The Queen's University of Belfast

CHAIR OF ANATOMY

Applications are invited for a Chair of Anatomy from 1 October 1984 or such other date as may be arranged. Salary is £19,416 per annum or, if medically or dentally qualified £20,301 per annum with contributory pension rights under FSSU or USS; an additional payment may be made by the Eastern health and Social Services Board on a sessional basis for any hospital services undertaken over and above University duties.

Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. Closing date: 31 July 1984. (Please quote Ref 84/N.) (3084)A

WASHINGTON STATE UNIVERSITY

RESEARCH COORDINATOR

PhD in biochemistry or related areas to provide day-to-day guidance to a research group composed of post-docs, graduate students and technicians working on enzymology and molecular biology related to lipids and other natural products under the direction of P E Kolattukudy.

Send, by August 15, 1984, a complete CV and references to Director, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340. WSU is an Affirmative Action/Equal Opportunity employer.

(NW618)A

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF CHEMISTRY

TEMPORARY LECTURESHIP IN INORGANIC CHEMISTRY

Applications are invited for a temporary lectureship in Inorganic Chemistry, tenable for one year from 1 September 1984. The lecturer will be required to give lectures at both elementary and advanced levels in the undergraduate course, to take laboratory classes, and to undertake research in collaboration with an existing research group. Applications will be particularly welcome from candidates having research experience in organometallic chemistry.

The appointment will be made in the salary range £7,190 to £8,530 per annum (under review) on the lecturer's scale, with USS benefits.

Further particulars of this post may be obtained from the Chairman of the Chemistry Department, Professor P A H Wyatt.

Applications (two copies preferably in typescript) with the names of three referees should be sent to the Establishments Officer, The University, College Gate, St Andrews, Fife KY16 9AJ to arrive not later than 18 July 1984. (2990)A

THE ANIMAL VIRUS RESEARCH INSTITUTE

HEAD OF MOLECULAR BIOLOGY

Applications are invited for a post of Head of the Division of Molecular Biology, the appointment to commence 1 October 1984. The successful candidate would also become Head of the Department of Biochemistry, and would lead a vigorous team investigating the biochemistry and genetics of several important exotic animal diseases including foot-and-mouth disease, bluetongue, and African swine fever viruses.

Candidates should have a high academic research record in the molecular biology of viruses and proven skills of leadership.

Salary in scale of £16,343-£20,794 (Grade 6 previously known as Senior Principal Scientific Officer). Sick leave and non-contributory superannuation schemes. 25 days annual holiday. House may be available for suitable married applicant. The successful applicant may qualify for financial assistance with removal expenses. The AVRI is an equal opportunity employer.

Application forms from the Secretary, Animal Virus Research Institute, Pibright, Woking, Surrey GU24 0NF. Tel: 0483-232441. Closing date for applications 10th July, 1984. (2967)A

UNIVERSITY OF GLASGOW

BIOTECHNOLOGY UNIT

POST DOCTORAL BIOCHEMIST

required to work on a 3-year project funded jointly by SERC and Pfizer Limited, to investigate the molecular biology of oxytetracycline resistance in commercial strains of Streptomyces rimosus, which produce high levels of the antibiotic.

The project will characterize the levels of expression of genes related to antibiotic resistance, study their expression signals in high and low producing strains, and investigate the biochemical mechanism of resistance. Biochemical analysis of export of antibiotic out of the cell will also be undertaken.

The position provides an opportunity to work on a commercially relevant topic, in close association with an industrial group.

Candidates with a strong biochemical background but limited recombinant DNA experience are encouraged to apply. A molecular geneticist is already in place, and the biochemist will have the opportunity to acquire recombinant DNA.

Initial salary — £7,630 — £8,530. Starting date up to 1 October 1984.

Further details and applications to Dr I S Hunter, Institute of Genetics, University of Glasgow, Church Street, Glasgow G11 5JS. Telephone 041-339 8855 ext 7112. (2989)A

UNIVERSITY OF COLORADO
DEPARTMENT OF CHEMISTRY

PROFESSIONAL RESEARCH ASSISTANTS IN BIOLOGY AND BOHYDRATE CHEMISTRY

Positions (2) are available for a plant biologist and an organic (carbohydrate) chemist to participate in the purification, isolation and characterization of biologically interesting complex carbohydrates. Applicants should have the equivalent of a Bsc or MSc in Plant Biology, Biochemistry or Chemistry. Starting salary (between $14,000 and $17,000) will depend on experience. The Univ. of Colorado is an affirmative action/equal opportunity employer.

Applications should be sent to Alan Darvill or Peter Albersheim, Dept of Chemistry, Univ of Colorado, Campus Box 215, Boulder, Colorado 80309. The applications will be evaluated August 15, 1984, [illegible]gh later applications will be [illegible]dered if the positions have not [illegible]illed. (NW627)A

POSTDOCTORAL RESEARCH ASSOCIATE

Crystallographic study of phospholipase A_2 and presynaptic neurotoxins and related complexes. Salary $18,000-22,000 depending on qualifications.
Write or telephone Paul B. Sigler, Department of Biochemistry and Molecular Biology, The University of Chicago, 920 E. 58th Street, Chicago, Il 60637. 312/ 962-1087. (NW645)A

UNIVERSITY COLLEGE CARDIFF
DEPARTMENT OF BIOCHEMISTRY

RESEARCH ASSISTANT

Applications are invited for a Research Assistantship to work with Dr N J Russell on the mechanism of membrane changes in moderately halophilic bacteria during adaptation to altered salinity. The work will involve membrane and lipid isolation/characterisation, radiochemical enzyme assays and TLC/GLC analysis. Candidates should have graduated in biochemistry, microbiology or a closely related subject.

The appointment, which is for three years, is funded by a grant from the Science and Engineering Research Council, and there will be some collaboration with the University of Ottawa. Salary RA range 1B £6,310 — £7,190 per annum. Duties to commence 1st September, 1984.

Applications (2 copies), including curriculum vitae, together with the names and addresses of two referees, should be sent to the Establishment Officer, University College, P O Box 78, Cardiff CF1 XL. Closing date 16th July, 1984. Ref: 2794. (2921)A

UNIVERSITY OF WARWICK

TEMPORARY LECTURESHIP IN VIROLOGY

Applications are invited for a temporary lectureship in the Department of Biological Sciences available for up to five years from October 1984. The successful applicant will become a member of the Virus Research Group and will be expected to undertake research in some aspect of virology and to participate in the teaching programme of the department.

The research programme of the Group is focussed on the general and molecular biology of several RNA viruses, defective particle-mediated interference, and the regulation of interferon synthesis. The position provides a career development opportunity for a young postdoctoral scientist with interests in immunological and molecular approaches to control of virus infection. Applications are invited from persons with experience in any aspect of molecular biology or cellular immunology.

The initial salary will be within the first six points of the lectureship scale: £7,190 to £9,425 pa. Further particulars and application forms from the Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref No 46/A/84/0. Closing date for receipt of applications 20th July, 1984. (3001)A

UNIVERSITY OF DUBLIN
Trinity College

LECTURER IN LABORATORY ANIMAL SCIENCES/ MANAGER, WELLCOME RESEARCH ANIMAL LABORATORIES

Applications are invited for the above post at Trinity College, Dublin.
The successful candidate, who should be a veterinary surgeon, will be expected to be responsible for the management of the centralised laboratory animal facilities of the University. It is anticipated that the person appointed will become involved in research projects and in the teaching of Laboratory Animal Sciences to students.
Appointment will be made on the Lecturer scale, at a point to accord with qualifications and experience to date.

Application forms and further particulars relating to this appointment may be obtained from:
Establishment Officer
Staff Office
Trinity College
Dublin 2
Tel: 772941 ext 1775

The closing date for receipt of completed applications will be 13th July, 1984. (2985)A

CSIRO
AUSTRALIA
BIOCHEMIST
$A25,342- $A37,274
DIVISION OF PLANT INDUSTRY CANBERRA ACT

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7,500 staff, of whom some 2,900 are professional scientists. The Organization's research activities are grouped into five Institutes: Animal and Food Sciences, Biological Resources, Energy and Earth Resources, Industrial Technology and Physical Sciences. The CSIRO Division of Plant Industry is a member of the Institute of Biological Resources.

FIELD: Photosynthesis.

GENERAL: The Division conducts fundamental and applied research on problems relevant to plant biology and agricultural production. One research program is concerned with photosynthetic processes: the molecular organization and regulation of photosynthetic membranes, the mechanism and significance of C_4 pathway of carbon dioxide fixation and primary productivity in aquatic ecosystems.

DUTIES: To undertake research on the relationship between the molecular organization of chlorophyll-protein and electron transport complexes, thylakoid membrane structure and photosynthetic capacities in plants adapted to different light climates or stressed conditions. The aim is to determine what regulates and potentially limits photosynthetic yield as a prerequisite to understanding how crop production might be improved.

QUALIFICATIONS: A PhD degree or equivalent qualification in an appropriate field with relevant research experience.

TENURE: A term of three years. Australian Government superannuation benefits are available.

APPLICATIONS: Stating full personal and professional details, the names of at least two referees and quoting reference No A4715, should be directed to:

The Chief
CSIRO Division of Plant Industry
GPO Box 1600
CANBERRA ACT 2601
AUSTRALIA

By July 27, 1984.

CSIRO is an equal opportunity employer. (W1081)A

SEPARATIONS TECHNOLOGY

Amicon Wright Limited is a small, highly successful UK subsidiary of a major American group at the forefront of separations technology. Continuing growth in manufacturing and developing industrial chromatography equipment has now created a vacancy for an applications technologist.

We are looking for a person who can not only combine theoretical and practical ability, but also work in close collaboration with engineers in order to develop and evaluate new products. This is an ideal opportunity to contribute your own ideas within a lively and practical development team.

You will be qualified to degree or equivalent level with experience of high performance liquid chromatography systems, ideally within a commercial environment.

We are able to offer excellent career prospects allied to an attractive, negotiable salary, plus relocation assistance in appropriate cases. Candidates should send career details to:

Mrs S Creswick,
Amicon Wright Ltd.,
Upper Mill, Stonehouse,
Glos. GL102BJ.

(3063)A

amicon wright

Indexers

required for the

ZOOLOGICAL RECORD

Applications are invited for the post of Indexer.

Candidates should possess a degree in Zoology or an allied subject, where zoology forms part of the degree course. Some linguistic ability and/or previous indexing or abstracting experience would be an advantage.

The *Zoological Record* is an annual bibliography of the world's zoological literature, availble both in printed form and as an online database.

Duties of the posts, which are based in York, include scanning and indexing the literature to produce input for a computer based operation.

Salary starts at £5,600 for a 35 hour week. Other benefits include 4 weeks paid annual holiday, non-contributory pension and Company sick pay scheme.

Handwritten applications, giving full details of qualifications, career to date, referees, etc., should be sent, to arrive no later than 13 July, 1984, to:

Administration Manager

BIOSIS, U.K. LIMITED,

Garforth House
54 Micklegate, York, YO1 1LF.

BIOSIS

(3008)A

Scientist —

Division of Viral Products

Research on Hepatitis 'A' virus

We have a position for a post-doctoral scientist to join a small group working on the molecular and biological aspects of picorna viruses, including Hepatitis 'A' virus.

Candidates should have previous experience in viral immunology and it would be advantageous to have an interest in molecular genetics of viruses.

Salary is on a scale of £8,816 — 11,896 inclusive dependent on experience, qualifications and age. The post is supported by external funds and is available initially for 3 years.

For further details please contact the Personnel Officer, NIBSC, Holly Hill, Hampstead, London, NW3 6RB. Tel. 01-435 2232. Informal enquiries may be made to Dr G C Schild. Please quote Ref. No. SU/ VP/ 293. (3062)A

NIBSC

National Institute for Biological Standards and Control

Department of Veterinary Pathology, University of Edinburgh and the Moredun Research Institute, Gilmerton Road, Edinburgh.

Applications are invited for the following appointments to an A.F.R.C. Linked Research Group between the Departments of Veterinary Pathology, University of Edinburgh and the Moredun Research Institute, Animal Diseases Research Association, Edinburgh. The appointments are for five years.

The research programme is concerned with fundamental studies of the sheep immune system in relation to disease mdoels of persistent viral infections of sheep. The posts are located within the University Department but there will be frequent interaction with the Institute as dictated by the research programme. The appointees will work with experienced research workers in immunology and virology.

Appointments:

Research Fellow 1A — Postdoctoral cellular immunologist for studies on the in vitro analysis of lymphocyte and accessory cell function and immune regulation in vivo within lymphoid tissue (Ref. LRG31/1). Salary £8,080 — £9,875.

Research Fellow 1A — Postdoctoral immunochemist/biochemist for biochemical characterisation of lymphocytes and accessory cell membrane glycoproteins, biochemical aspects of antigen presentation using viral polypeptides (Ref. LRG31/2). Salary £8,080 — £9,875.

Research Technician — (TG4/5) with experience in cellular immunology for studies on lymphoid cell function and monoclonal antibody work. Salary on range £5,826 — £6,898 (under review) with placement according to experience and qualifications. (Ref. LRG31/3).

Research Technician — (TG4/5) with experience of biochemistry for immunochemical work on cellular and viral antigens. Salary on range £5,826 — £6,898 (under review) with placement according to experience and qualifications. (Ref. LRG31/4).

Technical Assistant — (TG2) for general laboratory duties connected with the above research programme. Salary £4,662 — £5,151. (Ref. LRG31/5).

All appointments carry full superannuation and National Insurance benefits.

Applications (curriculum vitaes, 2 referees) for posts LRG31/1 and LRG31/2 should be sent to the Secretary to the University, Old College, South Bridge, Edinburgh, from whom further particulars can be obtained. Applications for posts LRG31/3, 4 and 5 should be sent to the Personnel Office, 63 South Bridge, Edinburgh. Informal enquiries can be made to Professor I. McConnell, Department of Veterinary Pathology, Royal (Dick) School of Veterinary Studies, Summerhall, Edinburgh. Tel 031-667 1011 ext 5322. Closing date for applications 6th July 1984.

(2910)A

TRANSGENE

TRANSGENE, an independent genetic engineering company, carries out high level research oriented towards applications of medical and industrial importance.

TRANSGENE has an opening for an experienced scientist as

HEAD OF THE FERMENTATION LABORATORY

The work will be devoted to the optimisation of fermentation process involving recombinant strains and/or cell lines.

It will require a strong interface with the research groups within the company as well as a practical understanding of aspects relevant for industrial purposes.

Therefore candidates must possess a strong background in biochemical engineering and a good expertise in fermentation and recovery development covering a range of hosts.

Salaries will be competitive and commensurate with qualification.

Knowledge of French is not necessary.

STRASBOURG is a pleasant historic town, located in the heart of Europe, very close to Germany and Switzerland.

The laboratory is located next to the University campus and enjoys close contacts with well known groups in fundamental research.

For confidential consideration, send a cv, a list of publications and indication of salary requirements to:

TRANSGÈNE S.A.
Attn J.-P. LECOCQ
11, rue de Molsheim
67000 STRASBOURG
FRANCE
Tél: (88) 22 24 90

(W1070)A

UNIVERSITY OF BRISTOL
DEPARTMENT OF BIOCHEMISTRY
GRADUATE RESEARCH ASSISTANT

Applications are invited for the post of Graduate Research Assistant to work on a SERC supported project on the molecular biology of a transposable element of Drosophila melanogaster.

The post is tenable for 3 years starting from 1st September 1984 at a starting salary of up to £6745 per annum for candidates below age 27. Deadline for applications is 15th August.

A strong background in nucleic acids and/or genetics would be advantageous.

Applicants should send a CV and two letters of reference to Dr. B. Chia, Department of Biochemistry, Bristol University, BS8 1TH. (3025)A

IMPERIAL COLLEGE
POST DOCTORAL RESEARCH IN FUSION TECHNOLOGY

Imperial College is undertaking a first technical appraisal of the potential of the densez-pinch as a fusion power source. Applications are invited from suitably qualified candidates for post-doctoral posts, initially of two years' duration, in an interdisciplinary team involving the Electrical and Mechanical Engineering and Physics departments.

Principal research areas will be:
— pulsed power technology
— radiation transport and effects
— dynamics of liquid metals
— plasma simulation

Address applications and requests for further details to: Dr S Walker, Mechanical Engineering Department, Imperial College, London, SW7 2BX. (3049)A

UNIVERSITY OF LONDON
KING'S COLLEGE LONDON
DEPARTMENT OF PLANT SCIENCES

Applications are invited for the post of
POSTDOCTORAL RESEARCH ASSOCIATE

tenable for a fixed term of two years to work on a project entitled 'The Regulation of Biosynthesis of Isoprenoids in Plant Tissue Culture' funded by the SERC (Biotechnology Directorate). Applicants should have a PhD degree in an appropriate field, and specific experience in enzyme methodology. Salary will be on the Grade 1A scale commencing at £9716 including London Allowance (scale due for revision), and the post is available from 1 October 1984.

Further details can be obtained from B V Charlwood, Department of Plant Sciences, King's College London, 68 Half Moon Lane, London SE24 9JF to whom letters of application, including a curriculum vitae and the names of two academic referees, should be sent by 16 July 1984. (3019)A

UNIVERSITY OF READING
DEPARTMENT OF BOTANY
RESEARCH OFFICER

required to work with Dr R E Longton on a British Antarctic Survey (NERC) funded project on water and temperature relations of masses from maritime Antarctic cryptogamic fellfields for a fixed period of three years from September 1984.

Starting salary £6,310 pa (under review). Possibility of registration for a higher degree.

Apply quoting ref R32A for application form and further particulars to Personnel Office, University of Reading, Whiteknights, Reading RG6 2AH. (3069)A

BIRBECK COLLEGE
UNIVERSITY OF LONDON
GEOLOGY TECHNICIAN — GRADE 2

Varied duties involving preparation for classes, use of general laboratory techniques including thin section making. Minimum qualification Diploma of Advanced Geological Techniques. Excellent working conditions. Salary scale £5,912—£6,544 (under review) inclusive of London Weighting.

Apply, stating age, qualifications and experience to Assistant Secretary (Personnel) (N) Birbeck College, Malet Street, London WC1E 7HX or telephone 01-580 6622 ext 529 for application forms. (3064)A

UNIVERSITY OF ST ANDREWS
DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

Applications are invited from physically fit single males to work mainly overseas. The post, which is for three years, is funded by a grant from the Natural Environment Research Council Special Topics Programme for Antarctic Research and is to investigate "muscle energetics, metabolism and locomotion in Antarctic animals".

Candidates should hold a good honours degree in physiology, zoology or some other relevant biological science, and have a strong interest in comparative physiology/experimental biology. Training will be given in muscle physiology and biochemistry. Some previous experience with electronics and/or computing would be an advantage. The successful candidate will leave for the British Antarctic Survey base at Signy Island during early 1985 and return in Spring 1987.

Starting salary £6,310 per annum (under review) on the 1B scale for research staff.

Further details of this post are available from the Establishments Officer, The University, College Gate, St Andrews, Fife, KY16 9AJ, to whom applications (two copies) including a full curriculum vitae and the names of two referees should be sent to arrive not later than 20th July 1984. (3045)A

Post Doctoral, Research Scientist

Department of Haematology and Oncology

Royal Children's Hospital Melbourne, Australia

An opportunity exists to undertake a leading research role in the fields of childhood leukaemia and childhood solid tumours for a scientist with experience in this field. The research laboratory, part of the department of Clinical Haematology and Oncology undertakes the care of more than 400 children with a variety of leukaemias and solid tumours and is the paediatric centre for bone marrow transplantation and autologous bone marrow rescue. Development and research are well established in the department and a list of research activities and publications will be forwarded on request. The Royal Children's Hospital is a major Paediatric centre in Australia and very good scientific support facilities are available in virtually all disciplines including immunology and recombinant DNA technology. Joint research projects with the Walter and Eliza Hall Institute and the Ludwig Cancer Research Institute are well established. Technical help will be available and there is scope for training of doctoral fellows.

The successful candidate will be funded at the appropriate N.H. and M.R.C. scale for seniority and experience (range $A20,164.00-$A30,737.00). Funds are available for a three year appointment, however transport and removal expenses cannot be provided.

Written applications describing the candidates area of interest, expertise, the proposed research programme for the three years, a curriculum vitae and the names and addresses of at least two referees, should be addressed to Dr. H. Ekert, Department of Haematology and Oncology.

Applications close Friday, 20th July, 1984.

Dr. Barry R. Catchlove,
Chief Executive.

(W1093)A

0412

Royal Children's Hospital

Flemington Road, Parkville 3052, Victoria, Australia.

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF BOTANY

POSTDOCTORAL POSITIONS IN PLANT MOLECULAR BIOLOGY

Applications are invited for two postdoctoral research assistant positions to work on aspects of chloroplast molecular biology. One post, supported by the Agricultural and Food Research Council, is to work on the synthesis of components of photosystem I in higher plants. The other post, supported by the Science and Engineering Research Council, is to characterise genes for components of the cytochrome complex in higher plants. Both posts are for a period of three years from 1 October 1984. The salaries will be within the 1A Scale.

Applications with curriculum vitae and the names and addresses of two referees should be sent as soon as possible to Dr J C Gray, Department of Botany, University of Cambridge, Cambridge CB2 3EA, from whom further particulars may be obtained. (3065)A

MRC CLINICAL RESEARCH CENTRE
(NORTHWICK PARK HOSPITAL)
WATFORD ROAD, HARROW
MIDDX HA1 3UJ

TECHNICIAN

Applications are invited for the post of Technician in the section of Vascular Biology to work with a team of scientists studying the generation of actions of inflammatory mediators. This position will become available in September and the work will involve investigation of microvascular responses in animal models, study of leukocyte functions in vivo and in vitro, and radioimmunoassay of mediators. Experience with pharmacological techniques and animal handling would be an advantage.
Preliminary enquiries may be made by telephone to Dr. J. L. Gordon (01) 864 5311 Ext. 2635 or Dr. T. J. Williams (01) 405 3474 Ext. 100. Applicants should have HNC or Degree in a relevant subject. Salary will be within the range £6,114-£8,370 depending on age and experience.
Application forms may be obtained by writing to Miss J. Cottam, Personnel Section, quoting reference 136/2/1546. Closing date for applications 20th July, 1984. (3005)A

UNIVERSITY OF KEELE
DEPARTMENT OF BIOLOGICAL SCIENCES
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above position to work with Dr J. Mills on the mechanism and regulation of ATP synthesis in chloroplasts. Experience in either membrane bioenergetics or protein purifivation techniques is desirable. This is a SERC-funded position tenable for three years (one year in the first instance) from October 1st, 1984. Salary is in the 1A range, £7190-£8080.

Application forms and further particulars are available from the Registrar, University of Keele, Keele, Staffs ST5 5DG, to whom completed applications should be lodged not later than 1st August, 1984. (3090)A

LOUGHBOROUGH UNIVERSITY OF TECHNOLOGY

Lecturer in Electrochemistry

Applications are invited for this "new blood" appointment available from 1st October 1984. New developing areas of electrochemistry have been selected for support with special reference to the electrochemistry of new materials and the development of new techniques, especially in their industrial aspects. The appointment will complement the existing applied electrochemistry group.
Salary on scale £7,190-£14,125. Application forms and further details from Paul Johnson, Establishment Officer, ref.84/31 CM. Closing date for receipt of applications 10th September, 1984. (3012)A
Loughborough Leicestershire

RESEARCH ASSOCIATE POSITION AVAILABLE

Center for Blood Diseases, University of Miami School of Medicine.

Requires PhD in Immunology with proven background of independent investigation, specifically first-author publications in the field, and experience with flow cytometric techniques, in platelet and/or macrophage immunology and cell culture. Responsibilities to include research in autoimmune hematologic disorders (platelet antibody assays, drug binding to platelets, macrophage culture and functions) and in an animal model of immune thrombocytopenia. Minimum salary — $21,000. **Send resumé and three letters of reference to Department of Medicine (R-760), University of Miami School of medicine, PO Box 016760, Miami, FL 33101. Equal Opportunity/ Affirmative Action Employer.** (NW640)A

MRC CLINICAL RESEARCH CENTRE
(Northwick Park Hospital)
Watford Road, Harrow,
Middx HA1 3UJ
TECHNICIAN

A Technician will be required in the near future to work in our division of immunological medicine on a clinical study of patients with asthma. Applicants should be capable of working alone, partially unsupervised. Qualifications required are BSc or HNC plus a current driving licence. Salary will be within the range £6114-£8370 (including London weighting) depending on age and experience. **For application form and job description, apply in writing please to Miss B Shaw, Personnel Office, quoting ref 118/2/3975. Closing date for applications, 20th July 1984.** (3080)A

NORTH EAST THAMES REGIONAL CYTOGENETICS CENTRE
Queen Elizabeth Hospital for Children
Hackney Road,
London E2 8PS
BASIC GRADE CYTOGENETICIST

Applicants must have a 1st or 2nd Class Honours Degree in Biological Sciences with preferably some experience in Clinical Cytogentics.

Appointments will be on Whitley PTA Scales for NHS Scientists. Applications by letter, including curriculum vitae and the names of two referees to the Director not later than two weeks from the appearance of this advert.

NB Previous applicants need not re-apply. (3100)A

EAST DORSET HEALTH AUTHORITY
ROYAL VICTORIA HOSPITAL
MLSO HISTOLOGY

Training post/career opportunity for experienced MLSO, Science Graduate or young person with "A" levels (Chemistry/Biology) as a medical laboratory Scientific Officer in our Histology Department. The successful candidate will be encouraged to study for the "Special Examination" in Cellular Pathology, set by the Institute of Medical Laboratory Sciences.

Salary on entry at age 21 years: £4674-£4878 when state registered: £5491-£7747.

Further details from Mr R Ellins, Senior Chief MLSO Pathology Department, Royal Victoria Hospital.

Application form and job description from Personnel Officer, Royal Victoria Hospital, Shelley Road, Bournemouth, BH1 4JG.

Closing date 12th July 1984. (3087)A

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)
DEPARTMENT OF IMMUNOLOGY
LECTURER:

Applications are invited from Molecular Biologists with an interest in immunology/histocompatibility immunogenetics. This appointment will be funded initially by The Cancer Research Campaign of Great Britain.

The successful candidate will be expected to establish and develop a molecular biology subsection of a Cancer Research Campaign funded group already working on the biology, immuno-chemistry and molecular genetics of MHC gene products in malignancy.

Salary will be on the University of London scales at Lecturer grade, within the range £9,490 to £16,440 plus London Weighting of £807.

Applications to Professor H Festenstein, Department of Immunology, The London Hospital Medical College, Turner Street, Lond E1 2AD. (309

MRC RADIOBIOLOGY UNIT, HARWELL
TECHNICIAN — CHEMIST/BIOCHEMIST

A Technician is required to join a team investigating modifications induced in biological macromolecules by ionising radiation. The project involves the techniques of radiolysis, simple analysis and purification procedures, general physical-biochemical techniques and use of microcomputers. Qualifications required are a degree or HNC/HTEC in a relevant subject. Previous experience would be helpful but is not essential as full training will be given.

Salary, according to age and experience, on the scale £5491 to £7747 pa. The Unit is situated in pleasant rural surroundings convenient for Oxford. Hostel accommodation is available for single people.

Please send applications, together with full curriculum vitae and the names of 2 referees, to the Personnel Officer, MRC Radiobiology Unit, Harwell, Didcot, Oxon OX11 ORD. Interviews will be held in early August. Closing date July 20th. Ref EMF/PC. (3077)A

nature classified

LONDON OFFICE
Keith Walesby
4 Little Essex Street
London WC2R 3LF
Tel: 01 240 1101
Telex: 262024

NEW YORK OFFICE
Miss Gene Fein
15 East 26 Street
New York, NY 10010
Tel: (212) 689 5900

TORONTO OFFICE
Peter Drake
Associates
32 Front Street West
Suite 200, Toronto,
Ontario M5J 1C5
Tel: (416) 364 1623

UNIVERSITY OF STRATHCLYDE
DEPARTMENT OF BIOSCIENCE AND BIOTECHNOLGY

WELLCOME LECTURESHIP IN IMMUNOLOGY

Applications are invited from candidates under 35 years of age, who do not hold a permanent appointment in a UK University for a Lectureship in Immunology, funded for up to 5 years by the Wellcome Trust, and thereafter by the University.

Candidates should possess expertise in biochemical immunology; immunogenetics or immunopharmacology and will be expected to take an active part in both undergraduate and postgraduate teaching, and to participate in and extend established research programmes. Interaction with other disciplines, especially Pharmacy and Pharmacology will be encouraged.

Candidates should submit a 500 word summary of their proposed schemes for research.

Further particulars and application forms (quote 35/84) are available from Staff Office. McCance Building, 16 Richmond Street, Glasgow, G1 1XQ.

Closing Date for Applications: 20 July 1984. (3081)A

Coláiste na hOllscoile Corcaigh
University College Cork

RESEARCH SCIENTISTS FOR BIOTECHNOLOGY PROJECTS

Applications are invited for the following postdoctoral positions in the Microbiology Department:

(1) Molecular Biologist to work on the regulation of genes involved in carbon and energy metabolism in Rhizobia during the free living and symbiotic states. Experience in either molecular biology, genetics or physiology in bacterial system(s) desirable.

(2) Microbial Geneticist to join a programme aimed at exploiting beneficial bacteria for biological control of phytopathogens. Experience in microbial genetics/recombinant DNA technology desirable to work specifically on the genetics of siderophore synthesis in gram negative bacteria.

Positions tenable for up to 2 years from 1 October 1984. Salary scale 1R £7,250 to £11,000 per annum according to qualifications and experience.

Applications enclosing Curriculum Vitae and names of three referees should be sent to Dr Fergal O'Gara, Microbiology Department, University College, Cork, Ireland. (3061)A

OSTDOCTORAL RESEARCH ASSOCIATE

Synthesis and study of competitive inhibitors of phospholipase A_2 by both biochemical and crystallographic methods. Salary $18-22,000 depending on qualifications.

Write or telephone Paul B. Sigler, Department of Biochemistry and Molecular Biology, The University of Chicago, 920 E. 58th Street, Chicago, Il 60637. 312/ 962-1087. (NW647)A

UNIVERSITY
OF WARWICK
Olfaction Research Group

POSTDOCTORAL POSITION
on
Cytochrome P-450
Monooxygenases

Unusually high levels of this enzyme have recently been discovered in olfactory epithelium. We wish to know whether the olfactory enzyme has any unusual properties in comparison with the well-studied liver enzyme; whether the enzyme plays a role in the olfactory transduction process and what role the enzyme plays in the protection of olfactory tissue from damage by air pollutants.

A strong background in enzymology and related areas is required together with an interest in the special problems of the olfactory system. The laboratory has specialised facilities for investigating the transduction mechanisms.

The post is available immediately for up to two years. Starting salary on the fourth point (£8,530) on the Research Range IA scale: £7,190 — £11,615 pa, depending on age and experience.

Written applications should include a full curriculum vitae and the names of two referees and should be sent as soon as possible to Dr G H Dodd, Department of Chemistry, University of Warwick, Coventry CV4 7AL (from whom informal enquiries may be made — phone 0203 523011 ext 2234. (3000)A

RESEARCH POSTS IN

Medicinal Chemistry
Cellular Electrophysiology
Neuroimmunology
Neuropharmacology

Applications are invited for positions at Senior Scientist level, tenable from 1 January 1985.

The Institute will be housed in well-equipped and spacious new laboratory accommodation which is being built within the precincts of University College London, with which strong academic links will be forged. The scientific staff will be built up to 25–30 by 1987.

The Institute will embark on a programme of basic research in neurobiology, concentrating initially on mechanisms involved in nociception, with the long-term aim of developing new compounds of potential therapeutic value. Initially there will be six laboratory groups (Medicinal Chemistry, Biochemistry, Neuropharmacology, Cell Biology and Cell Culture, Immunology, Cellular Electrophysiology) which will collaborate on a range of individual projects.

Applicants for these positions should be 28–35 years old, with at least two years' post-doctoral experience. The research programme of the Institute will be interdisciplinary in its approach, and an ability to contribute original ideas and to collaborate effectively are particularly important.

Medicinal Chemistry

For this position a good knowledge of synthetic organic chemistry and an enthusiasm for active collaboration with biologists in the design and testing of new compounds are essential. Experience of working in the pharmaceutical industry would be an advantage.

Cellular Electrophysiology

This post will require the application of electrophysiological methods to the analysis of drug action at the cellular level. Candidates should have considerable experience of micro-electrode recording techniques or *in vitro* preparations, preferably with experience of patch-clamping.

Neuroimmunology

This post will entail the applications of immunological methods to the study of neurones in culture and *in situ*, as a means of investigating neuropeptides surface antigens and receptors. Previous experience in this area is less important than a strong background in biochemistry, cell biology and immunology, and an innovative approach to research.

Neuropharmacology

Expertise in electrophysiological recording methods *in vivo* and *in vitro* is required. The work will involve studying the effects of drugs on activity in specific sensory pathways in the peripheral and central nervous system.

Salary negotiable around £15,000 p.a. according to experience and assistance with relocation costs available if relevant.

Applications by 16 July 1984, including c.v. and names of two referees, to Professor H.P. Rang, FRS, Director, Sandoz Institute for Medical Research, Department of Pharmacology, University College London, Gower Street, London WC1E 6BT (Tel: 01-388 5314), from whom further information can be obtained. (2898)A

SANDOZ

SCIENTIFIC DIRECTOR

The Hellenic National Biotechnology Company "BIOHELLAS" S.A. is seeking an experienced individual to serve as its Scientific Director. Responsibilities will include co-ordination of R and D and industrial production programs as well as long range planning. The Scientific Director will work closely with the Company's Scientific Board and Managing Director and will be based in Athens. Qualifications include extensive and industrial research experience in biotechnology, chemistry or related sciences.

Interested individuals should contact:

BIOHELLAS S.A.

10, Parnithos St., 154.52 Palaio Psychiko
ATHENS, GREECE Telephone Nr.6474770-4

(W1086)A

A.F.R.C. ANIMAL BREEDING RESEARCH ORGANISATION

EMBRYO MANIPULATION

Applications are invited for a molecular biologist/biochemist/-embryologist to join a team investigating the transfer of genes in farm animals and their subsequent expression and inheritance.

Candidates should have a first or upper second class honours degree in Biochemistry, Molecular Biology or relevant biological science preferably with experience of micro-injection of emryos or tissue culture cells. Applicants should have a Ph.D. or a minimum of two or four years relevant post graduate experience for appointment at Higher Scientific Officer or Senior Scientific Officer respectively. Salary according to qualifications and experience on the scales £7,149 to £9,561 (H.S.O.) and £8,970 to £11,476 (S.S.O.).

Applications giving full curriculum vitae and names and addresses of two referees to: The Secretary, Animal Breeding Research Organisation, Kings Buildings, West Mains Road, Edinburgh, EH9 3JQ.

The Agricultural and Food Research Council is an Equal Opportunities Employer. (3042)A

SOUTH TRENT CYTOGENETICS SERVICE
CITY HOSPITAL

SENIOR CYTOGENETICIST

£9,010-£11,649 p.a. (under review)

Candidates should have at least 4 years experience in Clinical Cytogenetics and be familiar with the full range of modern cytogenetic techniques.

Application forms and further particulars are available from the Personnel Department, Unit H.Q., City Hospital, Hucknall Road, Nottingham. NG5 1PB. Telephone (0602) 625459 (24 hour answering service).

Closing date: 20th July, 1984. (3007)A

Nottingham Health Authority

ST. BARTHOLOMEW'S HOSPITAL MEDICAL COLLEGE

DHSS DEPARTMENT OF TOXICOLOGY

LECTURER IN TOXICOLOGY

Salary Scale £8,376 – £15,311

This new post is a senior position for a graduate in a biological discipline with extensive experience in toxicology, especially in its pathological aspects.

The Lecturer will help to organise and run the scientific and educational work of the DHSS Department of Toxicology, concentrating on histo-pathological studies, and on assisting with research into pathological mechanisms of toxicity. The person appointed will be responsible both for service and investigative studies, as well as for teaching, and must have had several years experience of toxicology at a senior level.

There will be many opportunities to take part in a broad range of laboratory work, to develop new techniques and to aid the development of a growing department.

A job description may be obtained by telephoning 01-606 7404, quoting reference JR 15.

Please send a full Curriculum Vitae, (6 copies please) with the names of two referees to The Dean of The Medical College, St. Bartholomew's Hospital, West Smithfield, London EC1A 7BE.

Closing date 20th July, 1984. (2991)A

SYRACUSE UNIVERSITY

POSTDOCTORAL RESEARCH ASSOCIATE

Study of platinum antitumor drug-DNA interactions using natural DNA substrates of defined sequence. Experience in nucleic acid biophysics and/or DNA sequencing-gel electrophoresis preferred.

Curriculum vitae, three letters of reference to J C Dabrowiak, Department of Chemistry, Syracuse University, Syracuse, New York, 13210. Closing date 8/1/84. Equal opportunity affirmative action employer. (NW628)A

UNIVERSITY OF CALIFORNIA, DAVIS

PLANT VIROLOGIST

Assistant Professor of Plant Pathology, 11-month tenure-track appointment to begin on or before January 1, 1985, with 25% teaching, 75% research: to participate in a graduate course in plant virology and other courses in plant pathology; to develop original research program on etiology, epidemiology and control of virus diseases of agricultural crops; to participate in mission-oriented research, University service and advising and training of graduate students.

Applicants should have a PhD in plant pathology or a related discipline and expertise in plant virology. Send: a) curriculum vitae, b) official undergraduate and graduate transcripts, c) list of official published work with reprints, d) statement of teaching and research interests, e) names and addresses of three references to: J M Duniway, Department of Plant Pathology, University of California, Davis, CA 95616. Telephone: (916) 752-0324/0301. Closing Deadline: October 1, 1984.

The University of California is an affirmative action/equal opportunity employer (NW638)A

RESEARCH SCIENTI

Perform research and study i... lv-ing molecular and genetic immune mechanism and the regulation o these mechanisms during the aging process; Using biochemical, im-munological and dilution techniques and genetic engineering biotechnology to produce data fo various studies in aging and cancer PhD in Molecular Biology wit background in microbiology. Mus have publications in immunology.

Salary $34,000/year for 4 hours/week. Job in L.A.

Send this ad and your résumé t Job 1528, P O Box 865, Sacramen to, Calif 95804 not later tha 7/13/84. (NW630)A

UNIVERSITY OF NEWCASTLE UPON TYN

DEPARTMENT OF BIOCHEMISTRY

TEMPORARY LECTURER

Applications are invited for the pos of Temporary Lecturer tenable fro 1st October 1984 for three years in th Department of Biochemistry. Th post has been created by the award c a Lister Fellowship for a five yea period to Dr S J Yeaman.

Candidates will be considered wit research interests in any field biochemistry where appropria facilities can be made available, an an outline of plans for researc should be included with application

Salary will be at an appropria point on the Lecturers' scale: £7,1 — £14,125 per annum (with initi salary no higher than £11,615 p annum) according to age, qualif cations and experience.

Further particulars may b obtained from the Senior Assista Registrar (Establishments) The University, 6 Kensi Terrace, Newcastle upon Tyne N 7RU, with whom applications copies), together with the names a addresses of three referees should lodged not later than 9th Augu 1984. Please quote reference N. (3056)A

Derbyshire College of Higher Education

RESEARCH ASSISTANT

BIOLOGY

The post is tenable for 3 years from October 1984 to work under the direction of Dr M Greenwood on the reproductive physiology of viviparous and ovoviviparous cockroaches.

Candidates should have or expect to obtain a first or second class honours degree in a biological subject. The successful candidate will be expected to register for a higher degree.

Salary: £4,200-£4485 (under review).

Application forms and further particulars from the Staffing Officer, Derbyshire College of Higher Education, Kedleston Road, Derby DE3 1GB, telephone Derby 47181, extension 22, to whom completed forms should be returned by Friday, 13th July 1984. (3030)A

University of Cambridge AFRC Unit of Invertebrate Neurophysiology and Pharmacology

Animal Health Biology Pfizer Central Research

Post Doctoral Research Fellow Invertebrate Neuropharmacologist

Applications are invited for a postdoctoral research position to study the pharmacology of invertebrate GABA receptors. The post is funded by an SERC Co-operative Research Grant and will involve both electrophysiology (voltage clamp and patch clamp) on identified neurones and biochemical (radiolabelled ligand binding) studies. The research will be carried out in Cambridge in collaboration with neuropharmacologists from the Invertebrate Pharmacology Group at Pfizer.

Applicants should possess or be about to submit for a PhD.

The appointment will be for three years from 1st September, 1984.

Further information may be obtained from the secretary AFRC Unit of Invertebrate Neurophysiology and Pharmacology, Department of Zoology, Cambridge University, Downing Street, Cambridge CB2 3EJ, U.K.

Applications including curriculum vitae and the names of two referees should be sent to the AFRC Unit Secretary at the above address. (3101)A

CHIEF, ENDOCRINOLOGY & Research Scientist needed to lead endocrine, metabolic nutrition reference lab section; requirements: PhD in Science or related field, 3 yrs of related research, 2+ yrs supervisorial exp in a lab setting. Job site: Los Angeles; salary $43-50K/yr. Send this ad w/your CV to Job 2509, PO Box 865, Sacramento, CA 95804 before July 19 (NW641)A

POSTDOCTORAL RESEARCH ASSOCIATE

Crystallographic and biochemical study of protein/nucleic acid interactions including *trp* repressor and tRNA/synthetase complexes. Salary $18,000-20,000 depending on qualifications.

Write or telephone Paul B. Sigler, Department of Biochemistry and Molecular Biology, The University of Chicago, 920 E. 58th Street, Chicago, Il 60637. 312/962-1087. (NW646)A

IMPERIAL COLLEGE

POST-DOCTORAL RESEARCH ASSISTANT — AUTOMATICALLY GUIDED VEHICLES

A post-doctoral research assistant is required to join a multi-disciplinary group investigating various aspects of automatically guided vehicles, including the problem of real-time control of a fleet of free ranging AGV's. The work is funded by industrial research contracts, and the appointment would be initially for a period of one year.

Amongst the topics studied are vehicle to controller communications, predictive computer simulation and conflict avoidance, and vehicle location. The actual area of research would depend on the interests and experience of the successful candidate.

Applications are invited from candidates with good first degrees in engineering, physics or mathematics, and relevant PhD.

Address applications and requests for further information to: Dr S P Walker, Mechanical Engineering Dept., Imperial College, London SW7 2BX (01-589 5111 ext 2470). (3072)A

UNIVERSITY OF LONDON

BRITISH POSTGRADUATE MEDICAL FEDERATION

THE CARDIOTHORACIC INSTITUTE

RESEARCH ASSISTANT/ TECHNICIAN

Applications are invited for the above post which is available for 2¼ years for work on the biochemistry of cardiac protein turnover. Applicants should have an appropriate degree or suitable laboratory experience. In addition, experience with heart perfusion and/or amino acid analysis would be an advantage. There may be an opportunity to register for a higher degree. The post is based in London, N1.

Starting salary on the base point of the Whitley Council MLSO grade currently £5,491 plus £1,042 London Weighting. The post is superannuable.

Applications quoting reference CMB2 (with daytime telephone number, if possible) to the Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP, to include two copies of full curriculum vitae and names and addresses and telephone numbers of two academic referees. Closing date 20 July 1984. (3052)A

ASSISTANT PROFESSORS (3)

DEPARTMENT OF SURGERY

WEST HAVEN VA

YALE MEDICAL SCHOOL

General Surgery: Applicant must have completed a fellowship in colo rectal surgery. Special interest is required in the development of an experimental surgery laboratory as part of an active clinical and investigative program in the areas of the neural and hormonal modulation of large bowel function. In addition, a strong background in endoscopic, diagnostic and therapeutic procedures is required.

General Surgery: A special interest is required in gastrointestinal and endocrine surgery as part of an active clinical and investigative program in the area of the endocrine and gastrointestinal systems. Research experience in the area of plasma peptide radio immuno assay and isolated organ perfusion systems is required. Competence in both upper and lower GI endoscopic, diagnostic and therapeutic modalities is necessary.

Vascular Surgery: Applicant must have completed a vascular surgery fellowship. A special interest is required in the pathogenesis of atherosclerosis and in particular its relationship to prostaglandic metabolism. A substantial commitment to the development of an investigative program relating to endothelial cell culture and seeding is required.

All candidates will be expected to devote 60% of their time to investigative efforts and their remaining time to clinical and teaching responsibilities. The position will require a full-time commitment to the Yale Department of Surgery at the West Haven Veterans Administration Hospital.

Submit a complete cv and detailed letters of recommendation to:

Irvin M. Modlin, M.D., Vice Chairman
Department of Surgery
Yale University School of Medicine
P.O. Box 3333
New Haven, CT 06510

Yale is an equal opportunity affirmative action employer. Application deadline is August 15, 1984. (NW643)A

PHYSICIST

M & D Technology Ltd. operates at the forefront of medical instrument technology and enjoys a worldwide reputation in the design and manufacture of Nuclear Magnetic Resonance Imagers. Working closely in association with Aberdeen University the company already employs over 30 people, mainly engineers and physicists; and further expansion is planned.

The physicist will be part of a research and development team working on new imaging techniques. This is an opportunity for a talented physicist with an interest in NMR. A good honours degree is essential and higher qualifications advantageous.

Write in confidence giving full C.V. to:

Managing Director,
M & D Technology Ltd.,
Unit 1, Whitemyres Avenue,
Aberdeen, AB2 6HQ. (3009)A

AQUATIC ZOOLOGIST

Required to join team of ecologists working on the 'Kennet and Avon Canal' and environs in Berkshire. Previous experience in the identification of freshwater invertebrates and fish survey techniques would be an advantage.

This is an MSC project, and to be eligible, applicants must have been unemployed for six months (age 18-24) or for 12 months (age 25+). CV's to J. Matthews, Kennet and Avon Canal Project, Aldermaston Wharf, Padworth, Reading RG7 4JH., by 20th July, 1984. (3035)A

MRC BRAIN METABOLISM UNIT
Edinburgh
Organic Chemist

The MRC Brain Metabolism Unit invites applications from suitably qualified chemists for the post of Organic Chemist with special expertise in the synthesis of neuropeptides and appropriate analogues. Candidates of immediate post-doctoral or equivalent status will be preferred. A period (minimum 3 months) of training in the peptide chemistry laboratory of Dr R.C. Sheppard (MRC Laboratory of Molecular Biology) may be offered to a suitable candidate who, although highly competent in organic chemistry, has no special expertise in peptide chemistry.

The person appointed will work in close association with the Molecular Biologists in the Unit who are interested in the mechanisms of synthesis, release and mechanism of action of neuropeptides in mammalian brain. The appointment will be short-term (3 years). Remuneration will be at an appropriate point on the scales for university non-clinical academic staff.

Further details can be obtained from the Director, Dr G. Fink, MRC Brain Metabolism Unit, Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ. Four copies of applications and the names and addresses of three referees to be sent to Mrs A. Milligan, Administrative Officer, MRC Brain Metabolism Unit, Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ by Friday, July 27. (3096)A

AFRC INSTITUTE OF ANIMAL PHYSIOLOGY
Babraham Cambridge CB2 4AT
HEADSHIP OF DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the post of Head of the Department in succession to Dr R M C Dawson FRS who retires in June 1984. The Greville Biochemical Laboratory was completed in 1971 and provides 2,230 m^2 of laboratory space with excellent facilities for work on farm animals. The present programme includes genetic manipulation of ruminal micro-organisms and cellular biochemistry. It is expected that the successful candidate will have an established reputation as a leader of research in some field related to molecular signalling in cells eg gene expression, cell differentiation, hormone targetting, antigen recognition, etc. Collaboration will be encouraged with other research at the Institute which includes reproductive biology, endocrinology, neuroscience, cell biology and immunology.

The appointment will be at the grade of Senior Principal Scientific Officer (£15,605–£19,317). Further information can be obtained from the Director, Dr B A Cross FRS. Applications should be sent to the Director by the 28th July 1984. (3070)A

HOWARD HUGHES MEDICAL INSTITUTE
RESEARCH ASSOCIATE/ POSTDOCTORAL FELLOW

MD or PhD sought for 8/1/84 to participate in studies on the role of insulin receptor tyrosine kinase and regulatory protein phosphorylation in insulin action. Experience preferred in the area of reconstruction of membrane proteins; cell fusion; protein chemistry or immunologic techniques.

CV and 3 references required. Salary $17,000 — $20,000/year, depending on experience.

Contact: J Avruch, Howard Hughes Medical Institute Laboratory, Diabetes Unit, Massachusetts General Hospital, Boston, MA 02114; or call: (617) 726-2874. (NW616)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE UNIVERSITY OF LONDON
DEPARTMENT OF HAEMATOLOGY
JUNIOR TECHNICIAN

Required for one year to work on a research project on iron chelators.

Salary on appropriate Whitley Council scale for Junior MLSO s, according to age and experience.

Application forms, which should be returned with the least possible delay, available from School Office, Room G650, RFHSM, Rowland Hill Street, London NW3 2PF, or telephone 794-0500 extn 4262. Please quote reference H/JT. (3034)A

ASSISTANT PROFESSOR OF PLANT PATHOLOGY UNIVERSITY OF CALIFORNIA, DAVIS
VEGETABLE PATHOLOGIST

To develop an original research program on etiology, epidemiology, and control of diseases of vegetable crops, including physiogenic diseases, and to teach graduate courses in diseases of vegetable crops and IPM. An 11-month tenure-track position available immediately, with 25% teaching and 75% research. Appointee will also participate in mission-oriented research, University service, and graduate advising/training.

Applicants should have a PhD in plant pathology or related discipline and expertise appropriate to the position. Send: a) curriculum vitae, b) official undergraduate and graduate transcripts, c) list of official published work with reprints, d) statement of teaching and research interests, e) names and addresses of three references to: R N Campbell, Department of Plant Pathology, University of California, Davis, CA 95616. Telephone: (916) 752-0314.

Closing deadline: 17 September 1984.

The University of California is an affirmative action/equal opportunity employer. (NW637)A

UNIVERSITY OF BRISTOL
H.H.WILLS PHYSICS LABORATORY
RESEARCH ASSOCIATE IN THEORETICAL PHYSICS

Applications are invited for the [redacted] of Research Associate in Th[redacted] Physics, tenable for th[redacted] beginning as soon as [redacted]ssible. Preference will be given to applicants sharing research interests with members of the Theoretical Physics Group at the University of Bristol; these interests include condensed matter theory (R. Evans, D.A. Greenwood and B.L. Gyorffy), elementary particle theory (J.W. Alcock, W.N. Cottingham and B.R. Pollard) and nonlinear physics (M.V. Berry and J.H. Hannay).

Applications should be sent to The Registrar and Secretary, Senate House, Tyndall Avenue, Bristol BS8 1TH by 15th September 1984. (3024)A

UNIVERSITY COLLEGE LONDON
DEPARTMENT OF SURGER[redacted]
SCIENCE GRADUATE

required to assist with a three year project to work on a new treatment of cancer using lasers and photosensitising drugs. Experience with histology an advantage. Work with patients may be involved. Salary scale £6,520-£7,471 per annum plus £1,042 London Weighting.

Applications in writing to: Personnel Officer (Technical Staff GS3), University College London, Gower Street, London WC1E 6BT. Closing Date: 12 July 1984. (3041)A

UNIVERSITY OF NEWCASTLE UPON TYN[redacted]
DEPARTMENT OF ORGANIC CHEMISTRY
DEMONSTRATOR

Applications are invited for [redacted] Demonstratorship in the Departmen of Organic Chemistry starting fron 1st September 1984. The appoint ment will be for 2 years 4 months Preference will be given to candidate who are about to obtain or hav recently obtained a PhD i Chemistry.

The duties of the post will includ assistance with undergraduat tutorials and demonstrating. Th successful candidate will work in th research group of Professor B Golding.

Salary will be at an appropria point on the Grade IB (Bar) scal £6,310 — £8,530 per annum according to age, qualifications an experience.

Further particulars may b obtained from the Senior Assista Registrar (Establishments) (FP University, 6 Kensington Te Newcastle upon Tyne NE1 7RU, w whom applications (3 copie together with the names a addresses of 3 persons to whom re erence may be made should be lodg not later than 13th July 1984. Plea quote reference N. (3057)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BOTANY

TEMPORARY LECTURER IN PLANT ECOLOGY

[illegible]ations are invited for the post of Temporary Lecturer in Plant Ecology for one year from 1 September, 1984 to assist with the teaching of a first year and Honours courses. Preference will be given to those with experience and interest in plant population and community ecology.

Salary £7,190 per annum.

Applications, together with the names of three referees, should be received not later than 13 July, 1984 by the Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/556/N. (3028)A

TECH UNIVERSITY OF MUNICH

[illegible]ARTMENT OF PHYSIOLOGY

POST-DOCTORAL RESEARCH POSITION

A two-year position (extendable) will be available in autumn 1984 to join my research (electrophysiology, patch-clamp) on the function of the Na, K-pump in single myocardial cells. Experience in cellular electrophysiology and/or in the biochemistry of Na, K-ATPase would be valuable.

Applications should be sent to Dr J Daut, Physiologisches, Institut der Techn Universität, Biedersteiner-Str. 29, D-8000 München 40, FRG. (W1082)A

[illegible]HE LONDON HOSPITAL MEDICAL COLLEGE (University of London)

RESEARCH FELLOW IN MOLECULAR BIOLOGY: BONE & JOINT RESEARCH UNIT

Applications are invited for a Research Assistant to study the molecular biology of the HLA associated susceptibility gene and gene product in Coeliac Disease. Previous practical experience with cDNA hybridisation or related techniques is essential. A working knowledge of protein chemistry immunology and/or tissue culture is desirable.

Part of the project will be carried out in the molecular biology laboratories of the Department of Immunology which is conducting similar studies in other diseases.

The work is funded by a 2 year grant from the Wellcome Trust. Salary £7,190 — £7,630 plus £1,186 London Weighting.

Applications with cv and names of two referees should be sent as soon as possible to Dr J A Sachs, Bone and Joint Research Unit, The London Hospital Medical College, Turner Street, London E1 2AD. (2996)A

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF PAEDIATRICS

Applications are invited for the post of

POSTDOCTORAL RESEARCH ASSISTANT

funded by a project grant from Birthright to Dr D J Hill and Professor R D G Milner to study the presence and action of peptide growth factors in the control of human fetal growth and differentiation. Applicants should have experience in tissue and cell culture techniques and in the characterization and measurement of peptide hormones. Tenable for 2 years from 1 October 1984. Initial salary £7,190 a year on Range IA. Applications, together with the names of two referees should be sent by 13 July 1984 to Professor R D G Milner, Department of Paediatrics, Children's Hospital, Sheffield S10 2TH, from whom further information can be obtained. Quote ref: R115/7. (3088)A

PHARMACEUTICAL Research Scientist In Irvine. Plan/design/conduct/direct & coord. devel. & eval. of new drugs & dosage forms for eye & skin care. Devel. physical-chemical characterization technologies for preformulation studies. Eval. instruments, equip. & processes involved in dosage form research & initial mfg. Requires: PhD Chem & 2—3 yrs. exp. Interdisciplinary bkgrnd to perform complex pharmaceutical research. Thorough knowl. physical/organic/inorganic, analyt chem. Theoretical expertise w/polymere synthesis, thermodynamics, macromolecules, drug delivery. $3,063/month. Send this ad & your resume to Job 4128, PO Box 865, Sacramento, CA 95804 not later than July 15, 1984. Equal opportunity employer. (NW625)A

THE UNIVERSITY OF ASTON IN BIRMINGHAM

DEPARTMENT OF CHEMICAL ENGINEERING

RESEARCH ASSISTANT

Applications are invited for the above post to work on a study of the dynamics of a solvent extraction process at the Atomic Energy Establishment, Winfrith, Dorchester, Dorset. The work will involve computer modelling, and dynamics and control studies on pilot plant. It is sponsored by the UK Atomic Energy Authority.

The appointment will be for a period of three years and the commencing salary will be £6310 per annum. The successful candidate would be expected to register for a higher degree. Applicants should have an appropriate honours degree.

Application forms and further particulars may be obtained from the Staff Officer (quoting Ref: 615/6), University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET. Tel: 021 359 3611 Ext 4564. Closing date for the receipt of applications is 13th July 1984. (3085)A

NEWCASTLE HEALTH AUTHORITY

[illegible]ified 15

ROYAL VICTORIA INFIRMARY

DEPARTMENT OF MEDICINE

Research Medical Laboratory Scientific Officer or Research Scientific Officer (Graduate)

Applications are invited from suitably qualified persons to work on a project concerned with the immunological aspects of eye disease in collaboration with a Research Group working on autoimmune diseases under the supervision of Dr P. Kendall-Taylor, Consultant Physician, with a special interest in endocrinology, at the Royal Victoria Infirmary. Candidates *must* have a good background in biochemistry and it would be advantageous to have had previous experience in immunochemistry.

Salary Scale: M.L.S.O. (State registered) £5,491 p.a. to £7,747 p.a.
Scientific Officer (Science Graduate) £5,681 — £8,349)

Applications giving full details, and the names and addresses of two referees, should be sent to the Unit Personnel Officer, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, quoting Ref No. 84/39. Closing date 9 July 1984.

As an economy measure, no acknowledgements will be sent. If no communication has been received by 27 July 1984, it should be assumed that the application has been unsuccessful. (3038)A

THE MEDICAL COLLEGE OF ST BARTHOLOMEW'S HOSPITAL

DEPARTMENT OF CHEMICAL ENDOCRINOLOGY

CELL BIOLOGIST/ BIOCHEMIST

Required to work on a project studying the heterogeneity of circulating growth hormone and prolactin in patients with pituitary tumours. Previous experience in tissue culture and RIA is essential. This post is available for 2 years starting immediately. Salary range £9,010 — £9,310 p.a. plus £1,042 p.a. London Weighting.

Applications with full curriculum vitae, together with names and addresses of two referees should be addressed to The Secretary, Medical College of St Bartholomew's Hospital, West Smithfield, London, EC1A 7BE quoting reference RES 15.

Closing date: 28th June 1984. (3047)A

Technician or Junior Technician

required at the Plasma Fractionation Laboratory, Churchill Hospital, Oxford, for work on production and development of preparations from human blood plasma for the treatment of patients suffering from defects of blood clotting.

Appointment to the Technician grade requires academic qualifications equivalent to HNC, H-TEC or B.Sc. in a relevant subject together with a minimum of one year's relevant working experience.

Applicants for a Junior Technician post should have a minimum of four O-levels at grade C or higher, which must include English language, mathematics, and at least one science subject (preferably chemistry).

Salary scales (under review):

Technician £5,255 — £7,413.
Junior Technician £2,889 at age 16; incremental scale to £4,442 with additional allowance for appropriate qualifications.

Write for application form to: The Secretary, Plasma Fractionation Laboratory, Churchill Hospital, Oxford OX3 7LJ.

Closing date: 16th July, 1984. (2988)A

...ND POLYTECHNIC

...Pharmaceutical Sciences

S...nent of Pharmaceutical Chemistry

...tions are invited from persons who already have or are to achieve this summer, a good Honours degree in ...rmacy, Biochemistry, Chemistry or a related appropriate ...bject, for posts available in the following areas:

RESEARCH ASSISTANT

Salary: £4,947 – £5,649 (under review)

Plant tissue culture – the tissue culture of medicinal plants and investigation of alkaloid biogenesis in plant tissue culture. The work will be directed by Dr T H Bowen in collaboration with Dr R MacLeod (University of Newcastle).

RESEARCH STUDENT

Funded by SERC grant

Drug design/drug targetting – for cancer and for tropical diseases (the latter being within the newly-formed Tropical Diseases Chemotherapy Research Unit).

Application forms and further details of the research projects available may be obtained from the Personnel Officer, Sunderland Polytechnic, Langham Tower, Ryhope Road, Sunderland SR2 7EE or telephone (0783) 76231 ext 11.

Any informal enquiries may be made to Professor J R Brown, Head of Department, Pharmaceutical Chemistry, tel (0783) 76191 ext 40.

Closing date: 12 July 1984. (3032)A

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF ELECTRONIC AND ELECTRICAL ENGINEERING

RESEARCH ASSISTANT

Applications are invited from Physicists, Engineers or Applied Mathematicians, preferably with research experience, to assist with a project on fundamental aspects of ultrasonic wave propagation and scattering.

The project will involve both theoretical work (using both finite element and finite difference techniques) and some supporting experimental studies of wave in complex layered structures.

Applicants should have an aptitude for research and a good degree or postgraduate qualification in an appropriate subject.

Starting salary will depend upon age and experience. Applicants should send their curriculum vitae and the names of two referees immediately to: Dr Leonard Bond, Dept. Electronic and Electrical Engineering, University College London, Torrington Place, London WC1E 7JE. (3071)A

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY (UNIVERSITY OF LONDON)

PHYSICS DEPARTMENT APPLIED OPTICS GROUP

LECTURESHIP

Applications are invited for this post. The appointee will be required to teach optics at postgraduate level and to build up an active research programme as well as to teach undergraduate physics. Candidates should have research interests in experimental work on optical instrumentation and/or optical system design: candidates with expertise in related areas, such as the design and construction of integrated optical devices, are also encouraged to apply.

Salary is in the range £8376 to £15311 per annum (under review), including London Weighting.

Applications, including a curriculum vitae, list of publications and the names of three referees, should be sent to Professor T W B Kibble, Imperial College, London SW7 2BZ, to arrive not later than 31st August 1984. Further details may be obtained from Professor J C Dainty at the above address. (3020)A

CAMBRIDGE RESEARCH BIOCHEMICALS LTD

RESEARCH TECHNICIANS

required to work on the synthesis of organic compounds in the fields of peptide and nucleotide chemistry. Candidates should have a degree in chemistry or the equivalent. Experience of practical organic chemistry is essential. The position offer excellent opportunities to join a rapidly expanding biotechnology company.

Salary according to qualifications and experience.

Apply in writing to:

Dr G P Priestley
Cambridge Research Biochemicals Ltd
Button End
Harston
Cambridge CB2 5NX.

(3031)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London)

POSTDOCTORAL RESEARCH ASSISTANT

required for approximately two and a half years to work with Dr J Monjardino and Dr H C Thomas within the Departments of Physiology and Medicine supported by a grant from the MRC. Project involving the study of the molecular biology of hepatitis B virus. Applicants should have experience in nucleic acid biochemistry and preferably in mRNA isolation and characterisation.

Salary on scale Research and Analogous 1A.

Further details are available from the School Office, RFHSM, Rowland Hill Street, London NW3 2PF or telephone 01-794 0500 ext 4262. Full curriculum vitae, including the names and addresses of two referees should be submitted by 13 July 1984. Please quote reference M/RA. (2998)A

UNIVERSITY OF DUNDEE

CHAIR OF BIOCHEMISTRY

Applications are invited for the Chair of Biochemistry which will fall vacant on 1 October 1984 as a result of the resignation of Professor P B Garland to take up a senior research post in industry.

Further particulars are available from The Secretary, The University, Dundee DD1 4HN with whom applications (10 copies, or if posted overseas one copy) containing full career details, a summary of research interests, and the names of three referees should be lodged as soon as possible. Please quote reference EST/18/84. It is intended that shortlisted candidates should be interviewed in Dundee before the end of September, 1984 (J). (3046)A

MRC RADIOBIOLOGY UNIT HARWELL

RESEARCH OFFICER

There is a vacancy for a Research Officer, with a degree/HNC/HTEC in biology or genetics, in a group working on mammalian genetics with particular reference to mutagenesis. The work is varied and includes recombinant DNA work, animal genetics, cytogenetics etc. The successful candidate should preferably have at least 2 years appropriate laboratory experience, and if not, will be appointed as a Technician, with consideration later for re-grading.

Salary, according to age and experience, on the scale £6204-£8126 pa for a Research Officer and £5,491-£7747 pa for a Technician. The unit is situated in pleasant rural surroundings convenient for Oxford. Hostel accommodation is available for single people.

Please send applications, together with curriculum vitae and the names of 2 referees, to the Personnel Officer, MRC Radiobiology Unit, Harwell, Didcot, Oxon OX11 ORD. Ref. MFL/SW. (3076)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London)

GRADUATE RESEARCH STUDENT

Applications are invited ... students expecting to gradua... 1984 with at least a 2i in Pharmacology, Physiology or related subject. The successful applicant will need to be confident in the use of quantitative approach. The research (supported by MRC project grant for a research assistant) involves investigation of the electrophysiology of neuromuscular transmission in a myasthenic disorder using modern micro-electrode techniques. The study is to be undertaken jointly by the Department of Pharmacology and Neurological Science during a three year period, starting October 1984, and the applicant will be registered for a PhD.

Full curriculum vitae including the names and addresses of two referees should be sent by 13 July 1984 to the School Office, RFHSM, Rowland Hill Street, London NW3 2PF (01-794 0500 ext 4262) from ... further details may be obtained. (2995)A

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

A POSTDOCTORAL RESEARCH ASSISTANT

is required for an initial appointment of two years to work on algorithm development and computer analysis of cardiovascular signals. The project is in collaboration within the Cardiac Department, The London Hospital. The successful candidate will be expected to have a background in electrical engineering or computer science with knowledge of on-line programming.

Salary on 1A scale £7,190 – £11,615 + £1,186 LA.

Applications to Dr R Kitney, Department of Electrical Engineering, Imperial College, SW7. (3040)A

UNIVERSITY OF BRISTOL

ORGANIC GEOCHEMISTRY UNIT SCHOOL OF CHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

GC-MS characterisation o... biological markers from mil... chemical degradation of coals.

An organic/analytical chemist ... sought to explore the use of mil... chemical degradation procedures t... release identifiable biological marke... compounds from coals, fo... comparison with those of othe... ancient sediments and of petro... m...

This SERC-financed post is on th... 1A scale, commencing at £7,190 p... for one year in the first instanc... renewable for up to three year... Applications to Professor ... Eglinton, FRS, School of Chemistry, University of Bristol, Bristol BS8 1T... (Tel 0272-24161, ext 623). (3044)A

SEMINARS and SYMPOSIA

HELICON SYMPOSIUM 1984
NUTRITION AND DISEASE: CANCER
September 14 & 15 1984
Hyatt Islandia Hotel, San Diego, California

TOPICS

Mutagens and Antimutagens
Selenium and Vitamin E
Activation and Deactivation of Carcinogens
Carotenoids and Retinoids
The Role of Different Lipids
Future Research Strategies

GUEST SPEAKERS

Bruce N. Ames
W. Robert Bruce
John Cairns
T. Collin Campbell
Clifford Grobstein
David F. Horrobin
Clement Ip
Norman Krinsky
Daniel Medina
Richard Peto
David M. Prescott
Bandaru S. Reddy
Miriam P. Rosin
Eli Seifter
Gerhard N. Schrauzer
Meir J. Stampfer
Charles A. Thomas, Jr.
Clifford W. Welsch

REGISTRATION FEE

Before August 1st: $200. After August 1st: $240.

Registration is limited; for information write to:
Nomi Feldman, Conference Coordinator,
3770 Tansy, San Diego, CA 92121 (NW633)M

LECTURES

SECOND ANNOUNCEMENT

16th Lunteren Lectures on Molecular Genetics

Strategies in Vaccine Development

Period: 2-5 october 1984.
Place: Lunteren, The Netherlands
Costs: Dfl.425,– Fee + Board and lodgings
Dead lines: Application Forms – August 15, 1984
Abstracts – September 1, 1984
Registration fee + cost of living – September 1, 1984
Information: Corporate Commmunication Department TNO
P.O. Box 297
Telephone 070-8144 81 ext. 411
Telex 31660 tnogv nl
2501 BD 's Gravenhage
Netherlands

(W1094)K

STUDENTSHIPS

UNIVERSITY OF READING

DEPARTMENT OF FOOD SCIENCE

POSTGRADUATE RESEARCH STUDENTSHIPS

Applications are invited for four postgraduate research studentships, from 1 October 1984. Two available for graduates in a relevant discipline (Food Science, Chemistry, Biochemistry, Microbiology, Psychology, etc) for research on a topic within Food Science (chemical, physical, microbiological, processing, sensory, etc). Two are AFRC CASE awards. One is to evaluate factors that may involve human food choice and to propose a generalised model of food acceptability (graduates in a discipline with a significant mathematical/statistical component, eg, Statistics, Psychology, Economics); the other is to synthesise compounds to reinforce coffee aroma and to design appropriate aroma delivery systems (Chemistry graduates).

Applications from graduates with Honours degrees (I and IIi), stating age and qualifications and naming two referees, to Professor H E Nursten, Department of Food Science, University of Reading, Whiteknights, Reading RG6 2AP. (2987)F

UNIVERSITY OF LEEDS

ASTBURY DEPARTMENT OF BIOPHYSICS

SERC RESEARCH STUDENTSHIPS

Applications are invited from candidates who hold at least an upper second class degree in biophysics, computational science, physical biochemistry, physics or chemistry, or a suitable combination, for two research students tenable from October 1984.

1. A CASE studentship in collaboration with the Hannah Dairy Research Institute for studies by X-ray crystallography, computer graphics and spectroscopy on the 3-dimensional structure and function of the milk protein β-lactoglobulin.

2. An Information Technology studentship to develop computer graphics as a means of display of molecular structure and molecular interactions and in the design of molecules that might act as enzyme inhibitors for potential use as drugs.

Applicants must satisfy the SERC residential requirements.

Applications must include a Curriculum Vitae and the names and addresses of two referees and should be made as soon as possible and at the latest by 15 July to Professor A C T North, Astbury Department of Biophysics, University of Leeds, Leeds LS2 9JT. Telephone: (0532) 431751 ext 6130. (3086)F

UNIVERSITY OF CAMBRIDGE AND MEDICAL RESEARCH COUNCIL

POSTGRADUATE STUDENTSHIP

Applications are invited from graduates who have a First or Upper Second Honours Degree in Biochemistry, for an MRC postgraduate studentship, tenable for 3 years from October 1984.

The topic for research will be the regulation of renal and hepatic hydroxylation reactions in the vitamin D steroid endocrine system which controls cell calcium transport. This project is part of a long-term study into the molecular function of vitamin D.

Applications including the names of two referees, a curriculum vitae, and a telephone number where the applicant can be contacted, should be sent immediately to the Dunn Nutritional Laboratory, Milton Road, Cambridge CB4 1XJ. Telephone: (0223) 63356. Ref. DF/2. (3014)F

UNIVERSITY OF EAST ANGLIA

SCHOOL OF CHEMICAL SCIENCES

RESEARCH STUDENTSHIPS IN CHEMISTRY

The following SERC CASE studentships leading to the degree of PhD are available from 1st October, 1984.

1. Preparation and characterisation of water soluble shift reagents for ^{23}Na and ^{39}K and their application to cation transport in biological systems. (Dr R B Homer and AFRC Food Research Institute, Norwich).

2. Magnetic and optical properties of haemoproteins, especially peroxidases (Dr A J Thomson and Unilver, Port Sunlight).

Applicants must hold or expect to gain at least a II(i) degree in Chemistry and should send a curriculum vitae, including the names and addresses of two referees, to the person named at the School of Chemical Sciences, University of East Anglia, Norwich NR4 7TJ. (3068)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF GENETICS

An NERC RESEARCH STUDENTSHIP

is available starting October 1984 to investigate the genetics and physiology of tolerance to toxic heavy metals in bacteria from polluted aquatic environments.

The project will be supervised by Professor D A Ritchie and Dr P Strike, and would be suitable for graduates with a first or upper second class degree in Genetics, Microbiology, Biochemistry or a related biological science.

Gene-cloning techniques would be an important component of this study. Further information is available from Professor D A Ritchie (051-709 6022 ext 3114), and applications should be sent as soon as possible to: The Registrar, The University, PO Box 147, Liverpool L69 3BX. Quote Ref: RV/552/N. (2993)F

UNIVERSITY COLLEGE LONDON

RESEARCH STUDENTSHIPS

Two SERC Research Studentships leading to PhDs will be available for three years from October 1984 in the Department of Chemical and Biochemical Engineering on the following topics:

1 The use of immobilised cells for biochemical conversions of reactants dissolved in water-immiscible solvents (supervisor Prof M D Lilly).

2 The recovery and processing of rDNA polypeptide products from an aggregated form in E coli (supervisor Dr N M Fish).

Graduates in biochemistry, microbiology, biophysics, chemistry (protein chemistry), biochemical and chemical engineering interested in these topics should apply, and include a full curriculum vitae, to the appropriate supervisor in the Department of Chemical and Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE. (3094)F

continued on page 20, 21

STUDENTSHIPS

Southampton THE UNIVERSITY

Department of Biology

SERC RESEARCH STUDENTSHIP

Research in taxonomic databases

A studentship is available from 1st October 1984, for a graduate biologist to carry out research with Dr R J White and Dr F A Bisby on the application of computer databases in plant taxonomy. The project, will involve both conventional taxonomic practice and the use of computer methods currently being developed. The person appointed will be required to register for a higher degree. **Applicants, who should hold or expect to obtain a good honours degree, should write as soon as possible enclosing a curriculum vitae and the names and addresses of two referees to Dr R J White, Biology Department, Building 44, The University, Highfield, Southampton SO9 5NH, from whom further information may be obtained if required.** (3033)F

UNIVERSITY OF LONDON THE SCHOOL OF PHARMACY

TOXICOLOGY UNIT

SERC/CASE STUDENTSHIP

A SERC/CASE Studentship in collaboration with Imperial Chemical Industries is available from October 1984 leading to a Phd for research into the mechanisms of toxicity of chemicals and anti-cancer drugs. Applicants should possess or expect to obtain a good honours degree in Biochemistry or a related discipline.

Applications, including curriculum vitae and names of two referees should be sent by 16 July 1984 to: Dr G M Cohen, Toxicology Unit, Dept of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX. (3021)F

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)
Rowland Hill Street,
London NW3 2PF

Applications are invited for an

SERC STUDENTSHIP

to work on "Biophysical studies of cell membranes" under the supervision of Professor D Chapman. The project will involve the use of various spectroscopic techniques including laser flash photolysis and infrared spectroscopy. A good honours degree in biophysics, biochemistry or chemistry is required.

Applications together with names and addresses of 2 referees should be sent to Professor D Chapman, Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF by 13 July 1984. (2997)F

UNIVERSITY OF BIRMINGHAM

CENTRE FOR RUSSIAN AND EAST EUROPEAN STUDIES

CONVERSION COURSE FOR ENGINEERS AND SCIENTISTS

The Joint Committee of SERC/-ESRC has allocated a studentship for engineers and scientists who wish to extend their current interests into a social science field covered in the postgraduate training programme of the Centre for Russian and East European Studies. The coursework components include Russian language and Soviet studies.

The minimum qualification required for acceptance is normally a good second-class honours degree or equivalent in engineering, science or an applied science. Students graduating this year may also apply.

For further particulars and application forms please contact the Centre Secretary as soon as possible, by letter or telephone, at the Centre of Russian and East European Studies, University of Birmingham, P O Box 363, Birmingham B15 2TT, (021-472 1301 ext 2124). Please quote reference CCSS/Nl. (3048)F

ROYAL HOLLOWAY COLLEGE UNIVERSITY OF LONDON

SERC CASE STUDENTSHIPS

Applications are invited for two Science & Engineering Research Council CASE awards. (1) Physics — Project title; Microstructure of Dense Plasmas. Co-operating Body; Culham Laboratory, UKAEA. (2) Zoology — Project title; The Development of the Blood Fluke Sanguinicola inermis in Carp. Co-operating Body; Thames Water.

Applicants should be Physicists or Biologists/Zoologists who have achieved or expect to achieve, at least Upper Second Class Honours.

Details are available from the Registrar, Royal Holloway College, Egham Hill, Surrey TW20 0EX, to whom CV's and the names and addresses of two referees should be sent. (3074)F

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF MEDICAL CELL BIOLOGY

Applications are invited for a

RESEARCH STUDENTSHIP

tenable for 3 years commencing October 1984.

The project will be concerned with the uptake and intracellular processing of cell surface receptors and is part of a programme studying selective drug targetting. Techniques employed will include tissue culture, monoclonal antibody methods and electron microscopy.

Candidates should hold a first or good upper second class degree. They should apply in writing including the names of two referees to The Registrar, The University, PO Box 147, Liverpool L69 3BX. Quote Ref: RV/550/N. (2992)F

THE FOOD RESEARCH INSTITUTE

CHEMISTRY AND BIOCHEMISTRY DIVISION

SERC CASE STUDENTSHIP
Oxidative Stability of Lipids

Applications are invited for a SERC CASE Studentship tenable at the Food Research Institute and at the University of East Anglia's School of Biological Sciences to study the factors that limit the stability of commercially important fats towards oxidative degradation.

The project will involve the use of HPLC, GC, NMR and mass spectrometry to characterise and quantify the components in these fats that control oxidative stability and will also involve working for a part of the time at the Unilever Research Laboratory at Sharnbrook, Bedfordshire.

Candidates should hold, or expect to obtain, a First or Upper Second Class Honours Degree in Chemistry, Biochemistry or a related scientific subject.

Applications giving full cv and the names of two referees should be sent to: Dr H W-S Chan, Chemistry and Biochemistry Division, Food Research Institute, Colney Lane, Norwich NR4 7UA, by 12th July 1984.

We are equal opportunities employers. (3003)F

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF PHYSIOLOGY

RESEARCH STUDENTSHIPS

A three year MRC Studentship and an SERC CASE Studentship are available from 1st October 1984. The MRC studentship concerns the properties of primary afferent neurones innervating cranial blood vessels, the meninges and their possible involvement in migraine headache. The project will involve electrophysiological and histological techniques, and is under the supervision of Professor D I Wallis.

The SERC CASE studentship is concerned with the characterisation of 5-HT receptors in the CNS using electrophysiological methods in vivo, and is under the supervision of Dr M H T Roberts.

Applicants should possess, or expect to obtain a First or Upper Second Class Degree in Physiology, Pharmacology or some other appropriate subject.

Those interested should write as soon as possible to Professor D I Wallis or Dr M H T Roberts, Department of Physiology, University College, P O Box 78, Cardiff, CF1 1XL. Ref: 2798. (2940)F

INSTITUTE OF CANCER RESEARCH and ROYAL MARSDEN HOSPITAL

Sutton, Surrey

MRC STUDENTSHIP IN PHYSICS AS APPLIED TO MEDICINE

An MRC Research Studentship in Physics is available for the 1984/8 session, tenable for up to three years full-time study, leading, normally, to a PhD degree of the University of London.

The project will involve the development of high count rate detectors for improved medical imaging with particular application to the monitoring of therapy with radionuclides. The Physics Department has good electronic and mechanical workshops and extensive computing facilities and there is an active and growing research programme being carried out in the applications of Physics to Medicine.

Applications from candidates who hold or expect to gain a first or upper second class honours degree or a master's degree in Physics should be sent, together with a curriculum vitae and the names and addresses of two referees, to the Deputy Dean, Institute of Cancer Research, Block D, Clifton Avenue, Sutton, Surrey SM2 5PX (Tel: 01-643 8901, ext 296) as soon as possible. (3060)F

SCOTTISH CROP RESEARCH INSTITUTE

Invergowrie, Dundee DD2

RE-ADVERTISEMENT

(Previous candidates need not apply)

AFRC RESEARCH STUDENTSHIP IN PLANT VIROLOGY

Applications are invited for an Agricultural and Food Research Council studentship tenable for three years from October 1984 at the Scottish Crop Research Institute for research leading to a higher degree.

The project concerns the structure, antigenicity and properties of the particle protein of nepoviruses. The work will form part of a research programme on nepoviruses, which are important group of nematode transmitted plant viruses.

Applicants should hold a first or upper second class Honours degree in biochemistry, molecular biology or other relevant biological science.

Further details and application forms may be obtained from the Personnel Officer, Scottish Crop Research Instutute, Invergowrie, Dundee DD2 5DA. The closing date for applications is 20 July, 1984.

"The Institute is an Equal Opportunities Employer". (3091)F

STUDENTSHIPS

BIRBECK COLLEGE
(University of London)
ARCH STUDENTSHIPS
OLOGICAL AND
AL CHEMISTRY
plications are invited for two RC CASE Awards and one MRC rtnership Award. The projects volve drug design and metabolism, d disease diagnosis, especially ing NMR methods. There are portunities for travel and strong llaboration with clinical labor-ories. Applicants should have a ong chemical or biochemical back-ound and hold a first or upper ond class degree.

Please apply immediately and ange for a curriculum vitae and o references to be sent to Dr P J dler, Department of Chemistry, rbeck College, Malet Street, ndon WC1E 7HX. Telephone 580 6622 ext 262 or 326.
(3092)F

NIVERSITY OF
BIRMINHAM
Faculty of Medicine and
Dentistry
EPARTMENT OF IMMUNOLOGY
ND THE GENERAL HOSPITAL
PhD STUDENTSHIP
plications invited from suitably alified candidates (1 or 2.1 in bio-mistry or cell biology) for a 3 year D studentship funded by the ited Birmingham Hospitals dowment Fund. The project is to mine the origins and functions of teinase inhibitors in the human g with relevance to the patho-esis of acute and chronic lung eases. The work will involve cell ture, immunobiochemical and unohistochemical techniques. details may be obtained from urnett or Dr R A Stockley, nical Teaching Block, The neral Hospital, Steelhouse Lane, mingham B4 6NH. (3089)F

UNIVERSITY
OF NOTTINGHAM
School of Agriculture,
Sutton Bonington
DEPARTMENT OF
PHYSIOLOGY AND
ENVIRONMENTAL SCIENCE
MAFF RESEARCH
STUDENTSHIP
plications are invited from ents who hold or expect to obtain st or upper second class honours ee in Plant Science or a related ect, for a three-year studentship ing to a PhD. The project is on Physiology of pod-shatter in oil-rape.
details can be obtained J A Roberts, Department of siology and Environmental nce, School of Agriculture, on Bonington, Loughborough 2 5RD, to whom applications the names and addresses of two ees should be submitted by ly 1984. (2999)F

ROYAL HOLLOWAY AND
BEDFORD COLLEGES
AFRC CASE STUDENTSHIP
AFRC CASE Research Studentship is available to work on the modification of plant galactomannans by bacterial enzymes in collaboration with the British Food Manufacturing Industries Research Association. Applicants should have or expect to have at least Upper Second class Honours in Biochemistry, Chemistry or other Biological Sciences. Details are available from Professor J B Pridham, Royal Holloway College, Egham Hill, Egham, Surrey TW20 OEX. Telephone Egham 34455 to whom cv and the names and addresses of two referees should be sent. (3093)F

INSTITUTE FOR RESEARCH
ON ANIMAL DISEASES
POST-GRADUATE
STUDENTSHIP
Applications are invited from Biochemistry or Pharmacology honours graduates for a studentship, stipend £2,650 provided jointly by Hoechst UK and Roussel-Uclaf, which is tenable for three years. The successful applicant will join a team studying the pharmacokinetics and metabolism of drugs, particularly steroidal growth promoters. The appointee will be eligible to register as a PhD student.

The Institute is set in pleasant rural surroundings, with staff canteen and recreational facilities. Hostel accommodation available for single persons.

Further details and application form from: Secretary, Institute for Research on Animal Diseases, Compton, Newbury RG16 0NN, quoting ref: 519. Closing date for applications: 12th July 1984.
(3002)F

UNIVERSITY OF BRISTOL
DEPARTMENT OF PHYSICAL
CHEMISTRY
RESEARCH STUDENTSHIP
Appliations are invited for a research post to study two-phase flow in porous media, using a novel experimental technique. The project is funded by the Department of Energy, and forms part of a long-term study of problems related to Enhanced Oil Recovery. Requirements are a good first degree (BSc or equivalent) in chemistry, physics or petroleum engineering, and demonstrated experimental ability. The successful candidate will be expected to register for a higher degree. A maintenance grant (at the current SERC rate) and tuition fees will be paid. The appointment will be for one year initially, with possible extension up to three years.

Applications, giving details of qualifications and naming two referees, should be addressed to Dr J M Haynes, School of Chemistry, University of Bristol, Bristol BS8 1TS.

Closing date 3rd August, 1984.
(3006F)

CRANFIELD INSTITUTE
OF TECHNOLOGY
BIOTECHNOLOGY
CENTRE
Cranfield

RESEARCH STUDENTSHIPS

Applications are invited for the following studentships leading to the degree of PhD:

1) SERC studentship on quinoprotein based immunosensors, involving the study of bacterial glucose dehydrogenase as a label in conjugates with anti-Ig and its incorporation into biosensors.

2) Industrially funded studentship on the biodeterioration of tobacco, studying factors which may encourage fungal spoilage.

Applicants should have or expect a 1st or upper 2nd class honours degree, and should send a CV including the names of two referees to the Student Admissions Officer, Biotechnology Centre, Cranfield Institute of Technology, Cranfield, Bedford, MK43 OAL, from whom further details may be obtained.
(3036)F

DEPARTMENT OF BIOCHEMISTRY, UMDS (St. Thomas's)

Applications are invited from graduates in Biochemistry or related subjects to participate in research on DNA damage and repair in the ageing brain, based on an M.R.C. studentship.

Applicants would be expected to register for the degree of PhD, and should write to Professor H.S. Bachelard, Department of Biochemistry, St. Thomas's Hospital Medical School, London SE1.
(3039)F

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF BOTANY

RESEARCH STUDENTSHIP IN PLANT MOLECULAR BIOLOGY

Applications are invited for an SERC biotechnology Directorate Research Studentship to work on the characterisation of genes for ferredoxin and plastocyanin in higher plants. The studentship is tenable for three years from 1 October 1984. Applicants should possess a first or upper second class degree in a relevant biological subject.

Applications with curriculum vitae and the names and addresses of two referees should be sent as soon as possible to Dr J C Gray, Department of Botany, University of Cambridge, Cambridge CB2 3EA
(3066)F

OXFORD UNIVERSITY

Studentships in Atmospheric and Ocean Physics and Planetary Science

Two CASE studentships are available to read for the degree of Doctor of Philosophy (D.Phil.) commencing 1 October 1984.

Ship and Satellite Measurements of the Ocean Temperature: An infrared instrument of new design is to be built at Oxford and mounted on a ship of the British Antarctic Survey. During an eight-month voyage to the south polar cap, measurements will be made from the ship and from satellites as part of a study of ocean-atmospheric heat exchange and its role in the global climate. An NERC studentship is available to support an experimental physicist of robust constitution to participate in the project.

Dynamics of the Atmosphere of Jupiter: The Geophysical Fluid Dynamics Laboratory at the Meteorological Office is studying dynamical phenomena in the atmosphere of Jupiter by theoretical models and laboratory experiments. The Atmospheric Physics Department at Oxford is participating in the NASA Galileo Jupiter Orbiter mission to make spectral images of the atmosphere from which the structure and dynamics can be deduced. An SERC studentship is available for a physicist or applied mathematician to work on common aspects.

In each case applicants should hold or expect soon to gain a first degree of at least upper second class standard.

Please write to Dr. F. W. Taylor, Department of Atmospheric Physics, Clarendon Laboratory, Oxford OX1 3PU, with the names and addresses of two academic referees and a curriculum vitae.
(3015)F

FELLOWSHIPS

E M B O
European Molecular Biology Organization
LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY
AUTUMN 1984 AWARDS
Next deadline: August 15, 1984

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligibile a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are awarded initially for one year, but depending on a review of progress, they be renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is August 15, 1984. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on October 29, 1984.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F.R. Germany.

(W1016)E

MONASH UNIVERSITY
Melbourne, Australia

POSTDOCTORAL FELLOWSHIPS

Monash University is offering a number of Postdoctoral Fellowship positions commencing in 1985 and tenable for up to two years in any department of the University. Fellows will be expected to collaborate in their research with a member of the University staff.

Intending applicants should contact the Chairman of the Department in which they wish to work to discuss possible areas of mutual research interest.

Appointments will be in the Research Fellow Grade I range ($A20,991 — $23,436), but an appointment at Research Fellow Grade II level ($A24,353 — $31,994) may be considered in exceptional circumstances. Reference No 90162.

Applications will only be accepted on the official Application Form. This and other details are available from the Registrar, Mr J D Butchart, Monash University, Clayton, Victoria 3168, Australia. Applications close 28 September 1984.

An Equal Opportunity Employer.

(W1091)E

MASSEY UNIVERSITY
Palmerston North, New Zealand

DEPARTMENT OF ANIMAL SCIENCE

POSTDOCTORAL RESEARCH FELLOWSHIP IN PIG NUTRITION

Applications are invited for the above position funded by the New Zealand Pork Industry Board to work on an applied aspect of pig nutrition. Research experience in pig nutrition is essential and a particular interest in the nutrition of the young growing pig desirable.

The appointment is available from 1st October 1984 or soon after and will be for an initial period of one year. Remuneration will be NZ$21,600 per annum inclusive of an allowance of up to NZ$3,000 for fares.

Further details of the University, together with conditions of appointment, may be obtained from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF; or from the Registrar of the University with whom applications close on 3 August 1984.

(W1083)E

UNIVERSITY OF SURREY
AERE HARWELL

EXPLOITATION OF IMMOBILISED ANIMAL CELLS

Applications are invited for a postdoctoral research fellowship to study the effects of immobilisation on cultured animal cells. This work will be carried out at the University of Surrey as part of a larger programme funded by the Department of Trade and Industry.

The work will involve the culture of mammalian cells with particular emphasis on the design of suitable reactors and the study of the physical parameters so as to optimise the performance of such systems. Factors involved in the scale up of the equipment will also be investigated.

The Fellow will form part of a multi-disciplinary team which has been involved in the immobilisation and exploitation of eukaryotic cells for a number of years. In addition, both institutions are involved in other substantial industry oriented biotechnology programmes covering cell culture and product recovery.

The successful applicant is likely to be a chemical engineer, biochemist, cell biologist, or microbiologist with postgraduate experience in the growth and handling of tissue culture cells and a desire to apply scientific methods to the solution of technological problems.

The post will be for three years and salary will be in the range £7,190 — £8,975 per annum. Superannuation under USS conditions.

Applications in the form of a curriculum vitae (3 copies) including the names and addresses of two referees should be sent to the Deputy Secretary (Personnel), University of Surrey, Guildford, Surrey GU2 5XH, by 20 July 1984 quoting reference 282/N. Further particulars of the post can be obtained by telephoning Professor R E Spier (0483 571281 ext 687) at the University. (3053)E

THE AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH SCHOOL OF PHYSICAL SCIENCES

POSTDOCTORAL FELLOWSHIPS

Applications are invited from men and women who, at the time of application, have completed or expect soon to complete requirements for a PhD degree or equivalent qualification. Appointments are for two years only. They should be taken up during the first half of 1984 and may be made in any field of research of interest to the School. Departments of the School are: Applied Mathematics, Astronomy (Mount Stromlo and Siding Spring Observatories), Atomic and Molecular Physics, Engineering Physics, Mathematics, Nuclear Physics, Plasma Research Laboratory, Solid State Physics, Systems Engineering and Theoretical Physics.

Salary will be in accordance with qualifications and experience within the range: Postdoctoral Fellow Grade 1 (at a fixed point) $A21,4[illegible] — $A24,528 p.a. Current exchange rate: $A1 = 64p approx.

Grants are provided towards travel and removal. Assistance towards housing is given to an appointee from outside Canberra. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should write to the Acting Registrar of the University, GPO Box 4, Canberra ACT 2601, Australia, or the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, for further particulars before submitting applications which close on 31 July 1984.

The University is an equal opportunity employer. (3073)

THE UNIVERSITY OF LANCASTER
Department of Physics
RESEARCH FELLOWSHIP IN CONDENSED MATTER THEORY

A postdoctoral research fellowship is available for a condensed matter theorist to work on transport and scattering problems in ultra-low temperature physics. The position is for one year and the salary will be on the bottom point of the Research Range IA (£7190-£11615 - under review).

Further particulars may be obtained (quoting reference L078 from the Establishment Officer, University House, Bailrigg, Lancaster, LA1 4YW, where applications (six copies) naming three references, should be sent NOT LATER THAN 16 July 1984.

(3026)E

IMPERIAL CANCER RESEARCH FUND
Research Fellowship

to work on a collaborative project between the Royal Institution (Professor D. Phillips), Faculty of Clinical Sciences, University College (Dr. S. Brown) and the I.C.R.F. Laboratories, Lincoln's Inn Fields (Dr. I Hart). This interdisciplinary project on the role of phthalocyanines in photoradiation therapy of tumours, develop tissue culture and animal tumour model systems for evaluation uptake, selectivity of distribution and mode of action of a series of synthetic phthalocyanines. Experience in cell biology and/or pharmacology would be an advantage but suitable clinical background is also sought. The appointment is for 3 years and the work will be conducted in Dr. I Hart's laboratory at Lincoln's Inn Fields.

Salary range £8530 to £10710 plus £1250 London Weighting depending on qualifications and experience. Further details are available from the Personnel Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, W.C.2. Quote 82/84. Applications accompanied by names and addresses of three referees should be received by 18th August.

FELLOWSHIPS

[redacted]VERSITY OF SURREY — [redacted]ERE HARWELL

[redacted]OITATION OF [redacted]MOBILISED [redacted]NIMAL CELLS

Applications are invited for a

POST—DOCTORAL [redacted]ESEARCH FELLOWSHIP

study the effects of immobilisa- n on cultured animal cells. This rk will be carried out at the Aere- rwell as part of a larger pro- mme funded by the Department Trade and Industry.

The work will involve the culture of mmalian cells with particular em- asis on their immobilisation both hin and upon support matrices. e scale-up of such techniques and dies on their suitability for in- trial application will be under- en.

Fellow will form part of a multi- inary team which has been in- he immobilisation and ex- itati of eukaryotic cells for a nber years. In addition, both itutions are involved in other sub- ntial industry oriented biotechno- y programmes covering cell ture and product recovery.

The successful applicant is likely to a chemical engineer, biochemist, biologist, or microbiologist with tgraduate experience in the wth and handling of tissue culture s and a desire to apply scientific hods to the solution of technolo- l problems.

he post will be for three years and ry will be in the range £7,190 — 975 per annum. Superannuation er USS conditions.

pplications in the form of a cur- um vitae (3 copies) including the and addresses of two referees be sent to the Deputy (Personnel), University of rey, Guildford, Surrey, GU2 1, by 20 July 1984 quoting rence 281/N. Further particulars he post can be obtained by phoning Dr C Lambe (0235 24141 822) at Harwell. (3055)E

UNIVERSITY OF SURREY AERE HARWELL

EXPLOITATION OF IMMOBILISED ANIMAL CELLS

Applications are invited for a postdoctoral research fellowship to study the effects of immobilisation on cultured animal cells. This work will be carried out at the University of Surrey as part of a larger programme funded by the Department of Trade and Industry.

The work will involve the culture of mammalian cells with particular emphasis on the development of the cell biology and the nutritive medium so as to optimise the productivity of the system. This work would be expected to lead into the investigation of techniques for the downstream processing and recovery of the product.

The Fellow will form part of a multi-disciplinary team which has been involved in the immobilisation and exploitation of eukaryotic cells for a number of years. In addition, both institutions are involved in other substantial industry oriented biotechnology programmes covering cell culture and product recovery.

The successful applicant is likely to be a chemical engineer, biochemist, cell biologist, or microbiologist with postgraduate experience in the growth and handling of tissue culture cells and a desire to apply scientific methods to the solution of technological problems.

The post will be for three years and salary will be in the range £7,190 — £8,975 per annum. Superannuation under USS conditions.

Applications in the form of a curriculum vitae (3 copies) including the names and addresses of two referees should be sent to the Deputy Secretary (Personnel), University of Surrey, Guildford, Surrey GU2 5XH, by 20 July 1984 quoting reference 283/N. Further particulars of the post can be obtained by telephoning Professor R E Spier (0483 571281 ext 687) at the University. (3054)E

ASSISTANTSHIPS

UNIVERSITY OF OXFORD
Department of Theoretical Physics

POSTDOCTORAL RESEARCH ASSISTANTSHIP IN STATISTICAL AND ATOMIC THEORY
(in association with the Theoretical Physics Division, AERE Harwell)

Applications are invited for a postdoctoral Research Assistantship to work jointly in Oxford and at Harwell in areas of research of mutual interest. This temporary appointment is for one year in the first instance but normally extendable for a further period. The position is available from 1 October 1984. The salary is on the Research Staff Scale 1A, presently £7,190-£11,615 (with USS membership).

Applications with names and two referees to Professor R J Elliott, Department of Theoretical Physics, 1 Keble Road, Oxford OX1 3NP by 31 July 1984. (3082)P

UMIST

RESEARCH ASSISTANT IN PHYSIOLOGICAL PSYCHOLOGY OF VISION

Applications are invited from honours graduates in science with an interest in visual physiology for the post of Research Assistant to study stability of colour and pattern perception and cognitive performance of human observers.

The project will involve psychophysical, electrophysiological (visual evoked potential) and psychopharmacological studies, mainly on human observers, but may be extended to studies on monkeys. It can lead to higher degrees (MSc or PhD).

The appointment on this sponsored project will be initially for a period of 12 months. Salary will be according to age, qualifications and experience on the scale £6310-£8530 pa (under review).

Applications and enquiries, quoting reference OP/103, including a curriculum vitae and the names and telephone numbers of two referees, should be addressed to Professor Cronly-Dillon, Visual Sciences Lab, Department of Ophthalmic Optics, UMIST, PO Box 88, Manchester M60 1QD (tel 061-236 3311 ext 2521). (3083)A

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

OPTICS SECTION

POSTDOCTORAL RESEARCH

New research programmes in image processing using four wave mixing in photorefractive media, space-time photon correlation, astronomical image reconstruction and coupling into optical waveguides using diffractive elements have been established. There are excellent laboratory facilities for these projects.

Several postdoctoral research assistantships are expected to be available starting between October 1984 and January 1985 for periods of 1-2 years. Salary in the range £8,376 — £12,801 including London Allowance (under review).

Enquiries and applications (cv and names of two referees) to Prof J C Dainty, Blackett Laboratory, Imperial College, London SW7 2BZ before 20th July 1984. (3051)P

AWARDS

IMPERIAL COLLEGE AND THE MRC CLINICAL RESEARCH CENTRE

ORGANIC CHEMISTRY AND CLINICAL CELL BIOLOGY

MRC PARTNERSHIP AWARD

Applications are invited for an MRC partnership award leading to a PhD degree. on the synthesis and biological activity of R-glutamyl prodrugs for use in cancer chemotherapy. The research will involve the synthesis of novel organic compounds, examination of their stability and activation by enzymes and assessment of their cytotoxic activity. It will provide broad training in organic synthesis and enzymology as well as cancer studies. The project will be supervised by Dr B C Challis (Imperial College) and Dr T J Peters (Clinical Research Centre) with equal time spent at each institute.

Applications with curriculum vitae and the names of two referees should be sent to Dr B C Challis, Department of Chemistry, Imperial College, London SW7 2AY. (3050)N

MASSEY UNIVERSITY
[redacted]nerston North, New Zealand

POSTDOCTORAL FELLOWSHIPS

lications are invited for doctoral Fellowships in 1985, to unded and awarded by the ersity Grants Committee and le within Massey University.

ards may be made for one year r three years.

tions from candidates work in any department of sity will be considered, but e awards the University ittee will give priority research areas such enetic engineer- engineering,

The emolument payable will be at a rate to be determined but not exceeding the lowest step on the University scale for lecturers (currently NZ$21,600 per annum).

Applicants should have completed the requirements for the award of the degree of Doctor of Philosophy.

Application forms together with the general conditions of appointment may be obtained from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF; or from the Registrar of the University with whom applications close on 31 July 1984. (W1092)E

THE UNIVERSITY OF AUCKLAND

DEPARTMENT OF CELL BIOLOGY

RESEARCH FELLOWSHIP

A Fellowship is available for a PhD graduate to work on the effect of cyclic AMP and calcium on protein kinases in tumour cells and the control of tumour cell growth. Applicants should have a relevant background in biochemistry, cell biology, or molecular biology. An appointment will be made for one year initially with the possibility of renewal for a second year.

Further information is available from Professor R K Ralph, Department of Cell Biology, University of Auckland, Private Bag, Auckland, New Zealand. Applications will close on 27 July 1984. (W1089)E

continued on page 24

Advertisement

PFIZER AWARDS — 1984

In October, 1982 Pfizer Central Research at Sandwich, Kent announced that they would make up to six new Awards annually of £1,500 each to support young scientists who had carried out meritorious research at British Universities. The research should have potential application in the search for human or animal health drugs.

The awards are made by a panel of Pfizer scientists in consultation with senior academic advisers, on the basis of work published in the two years prior to the award. The awards are NOT open to application. Each award may be renewed at the panel's discretion for a second year. The awards are for the personal use of the recipients to further their research.

The 1984 awards are:

Chemistry

Dr. G.W.J. Fleet, Dyson Perrins Laboratory, University of Oxford. *"For his enantiospecific total syntheses of shikimic acid and pseudomonic acids from carbohydrates."*

Dr. J.K.M. Sanders, University Chemical Laboratory, Cambridge. *"For his work on the application of revolutionary new NMR techniques to the determination of the preferred solution conformation of complex natural products".*

Biology

Dr. T.J. Williams, Institute of Basic Medical Sciences, Royal College of Surgeons, London. *"For his work on the interaction of mediators in the inflammatory process".*

Dr. H.J. Field, Department of Clinical Veterinary Medicine, University of Cambridge. *"For his contribution to our understanding of the pathogenesis of recurrent herpes virus infection".*

Pharmaceutical Sciences

Dr. P. York, School of Pharmacy, University of Bradford. *"For his research into the solid state properties of powders and related areas of phamaceutical technology".*

Pfizer is a worldwide, research-based company with inter-related businesses in health care, agricultural, specialty chemicals, materials science and consumer products. The Company has a major commitment at Sandwich where it employs approximately 1,500 people, over 500 of whom are engaged in new product research. (3029)N

GRANTS and SCHOLARSHIPS

CIBA FOUNDATION SYMPOSIUM BURSARY SCHEME

Aim: To enable young scientists to attend Ciba Foundation symposia and subsequently spend 4 weeks in the laboratory of one of the symposium participants.

The bursary will cover —

a) travel expenses
b) board and lodging for the duration of the bursary

Qualifications: Applicants must be aged between 25-35 years on the closing date for application, and actively engaged in research on the topic covered by the symposium of their choice.

Topics:
1 GROWTH FACTORS IN BIOLOGY AND MEDICINE
2 MELATONIN AND THE PINEAL
3 MOLECULAR BIOLOGY OF MACROPHAGE DIFFERENTIATION
4 IMMUNOLOGY OF PEPTIDES

Applications: In writing to:
The Director, The Ciba Foundation, 41 Portland Place, London W1N 4BN
including the following information:
a) Full name, address and date of birth
b) Symposium of choice (one from the above list)
c) Qualifications and short resumé of further education
d) Career history, including full list of publications
e) Details of current research and aims of future career
f) Names and addresses of two referees

Closing Date for Applications: 30 September 1984 (3022)H

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THE MOLECULAR BIOLOGY OF CANCER

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NATURE Magazine
15 East 26 St., New York, NY 10010

Please do not telephone. Write, including a brief synopsis of poster subject and its scientific relevance. Poster space is limited, so we suggest you act promptly.
(NW468)

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London WC1R 4EB and The William Byrd Press Inc., Richmond, Virginia, USA. Published by MACMILLAN JOURNALS LTD, 4 Little Essex Street, London WC2R 3L

SCANNING ELECTRON MICROSCOPY at MICRO 84

84 takes place at the Bloomsbury Crest Hotel in London from July 9th to 13th 1984. MICRO is the largest conference and exhibition concerned with all aspects of light and electron microscopy; and any one microscopical technique, such as SEM, can be easily seen in the context of all the other, allied techniques, available. Today, the SEM is very much a microscopist's workhorse and there is a strong SEM theme running through the whole of MICRO. Some symposia are directed specifically to SEM, others have particular lectures which are relevant to SEM, while in still others complementary techniques are discussed. The following list highlights some of these lectures.

SEM OPIMISATION
(Thursday/Friday)

The main objective of this symposium is "Getting the best out of your SEM". A series of tutorial lectures from acknowledged experts will cover the instrument, its operation, specimen preparation, image recording and image interpretation.

TUTORIALS IN MODERN ASPECTS OF MICROSCOPY
(Wednesday)

This Symposium is concerned with microscopy and analysis of solid specimens. The lectures will not only cover SEM methods for the non-expert, but also Quantitative Microscopy, Scanning Optical Microscopy, Acoustic Microscopy, and Atom Probe Analysis.

STATE OF THE ART IN TEM AND STEM
(Tuesday)

A series of tutorial lectures will deal with imaging and analysis of thin specimens. Among other things, the symposium is intended to update the knowledge of those not working directly with TEM or STEM.

ANALYSIS IN THE SEM
(Friday)

In the sessions on X-ray microanalysis and cryo-techniques a number of the presentations specifically deal with preparation of specimens, including by low temperature methods, and their analysis in SEMs.

IMAGE RECORDING
(Tuesday)

The symposium will include lectures on optimisation of scanning images, electronic processing of images, and photographic recording of images.

APPLICATION OF SEM
(Throughout MICRO)

Throughout MICRO, many of the sessions will include lectures on applications of the SEM to structural observations and microanalysis. Topics including an SEM component include: interfaces in semi-conductors; automated SEM in environmental studies; automatic SEM particle analysis in forensic science; SEM in the study of ancient materials.

EXHIBITION
(Throughout MICRO)

It is expected that more than 50 companies will be exhibiting their latest microscopes and ancillary equipment, covering the complete spectrum of instruments and techniques including, of course, full representation of SEM equipment.

Details of the MICRO 84 programme, and further information, are available from:-

The Administrator, Royal Microscopical Society, 37/38 St Clements, OXFORD OX4 1AJ. Telephone: (0865) 248768/721081

Women Scientists

The Road to Liberation

Edited by **Derek Richter**
Institute of Neurology, University of London

April 1982 £10.00 313pp ISBN 0 333 32468 4

Anyone should find these glimpses of lives in other cultures intriguing."

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This book draws together the personal and intellectual biographies of eleven women biomedical scientists. The individual stories are informative, fascinating, and sometimes moving, and cover cultures as diverse as those of India, Iran, USA and the USSR.

Please send orders or requests for further information to Carolyn Leuw
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THE MOLECULAR BIOLOGY OF CANCER

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